ANIMAL VIRUS GENETICS
Bernard Fields and Rudolf Jaenisch, Organizers
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Plenary Sessions

Workshops & Poster Sessions

Genetic Interactions Between Viruses

586 THE INSERTION SEQUENCE IS2 - A PROKARYOTIC CONTROLLING ELEMENT, Heinz Saedler, Debabrota Ghosal, Hans Sommer, John Cullum and Peter Peterson, Institut für Biologie III, Schänzlestr. 1, 78 Freiburg i. Br., West Germany.

The insertion sequence IS2 is a transposable element in Escherichia coli K12 which seems to integrate preferentially into promoter-operator regions. Integration in one orientation (Orientation II) leads to constitutive expression of downstream genes from a promoter on IS2. Integration of IS2 in the other orientation (Orientation I) normally leads to turn off of downstream genes due to a rho-dependent transcriptional termination signal on IS2. However, DNA rearrangements in IS2 can give rise to new promoters. These promoters can be stable or have varying degrees of instability. DNA sequence analysis of these rearrangements suggested models for the formation of some of these promoters and for their instability (1,2). IS2 can also promote alterations in neighbouring DNA sequences such as deletion (3). Integration of one type of insertion sequence into another (such as IS2 into IS1, or IS3 into IS2) may also lead to alterations in the expression of neighbouring genes (4). Thus, IS2 can be considered as a controlling element in E.coli K12 with a considerable range of effects on gene expression.

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587 MOLECULAR GENETICS OF HEMOPOIETIC DISEASE INDUCTION BY HELPER-INDEPENDENT AND REPLICATION-DEFECTIVE FRIEND TYPE-C RETROVIRUSES, E. M. Scolnick, D. Linemeyer, A. Oliff, S. Ruscetti, Tumor Virus Genetics Laboratory, National Cancer Institute, Bethesda, MD. 20205, and M. Dexter, Paterson Laboratories, Christie Holt and Radium Institute, Manchester, England.

Friend murine leukemia inducing virus stocks contain both helper-independent and replication-defective viruses capable of inducing erythroproliferative syndromes in susceptible mice. The helper-independent virus (F-MuLV) causes hepatosplenomegaly and anemia (hematocrit 20) in susceptible newborn mice. In the late stages of the disease the predominant cell in the spleen is a hyperbasophilic blast cell which looks like an erythroblast and the peripheral smear shows an apparent block in the terminal stages of erythroid differentiation. The replication-defective virus (SFFV) determines an erythroproliferative syndrome in susceptible adult mice in which the terminal steps of erythroid differentiation still occur and where hematocrits of over 55 are regularly seen. Each virus, F-MuLV and SFFV has been molecularly cloned in a fully infectious DNA form. By marker rescue studies, we have localized the region of F-MuLV responsible for its disease induction to a piece of DNA approximately 4.0 KB in size. This piece includes the entire 3' third of the 9.1 KB F-MuLV genome. In studies on SFFV we have obtained evidence that two molecularly cloned forms of retroviral proviral DNA can undergo recombination in mammalian cells, and we have defined two Eco R1 restriction sites which must be intact for the SFFV genome to induce spleen foci. Both Eco R1 sites map in the 3' half of the SFFV genome, and one site is located in the ENV gene of SFFV. Although the genetic analysis is not complete, the results suggest the validity of the approach to a rigorous definition of the disease-determining genes of these viruses.

588 A GENETIC ANALYSIS OF HERPES SIMPLEX VIRUS USING INTERTYPIC RECOMBINATION, Wilkie, N.M., Davison, A., Chartrand, P. and Crumpacker, C.

The DNA genome of herpes simplex virus (HSV) comprises two segments (L and S) which are bounded by inverted repeat sequences. A direct terminal repeat of 250-400 base pairs (the 'a' sequence) is also present at the 'joint' between the two segments. The L and S segments are found in inverted orientations, giving rise to four isomers present in approximately equal amounts in virion DNA.

The oral (HSV-1) and genital (HSV-2) serotypes of herpes simplex virus interact genetically at the level of both complementation and recombination (1,2). Intertypic and intratypic marker rescue (3,4) and phenotypic analysis of intertypic recombinants (5) have permitted the physical location of a number of conditional-lethal mutants and of genes coding for structural and non-structural polypeptides and enzymes. Our current data will be summarised and related to the lytic cycle.

Intertypic recombinants which are heterotypic for the repeat sequences which bound a segment, fail to isomerise that segment normally. Unlike others (6,7), we conclude that "obligatory copying" of the repeat regions (leading to isomerisation) is not an essential feature of replication.

Detailed restriction endonuclease mapping of sub-clones shows that the presence of homotypic 'a' sequences at each end of L is sufficient for normal isomerisation. A demonstrated region of highly conserved sequence homology in the S repeats of HSV-1 and HSV-2 is not sufficient to allow normal isomerisation of this segment, and the results suggest a sequence-specific mechanism involving the 'a' sequence. They further suggest that the previously reported 'obligatory identity' of parts of the repeat sequences (6,7) may be due to the observed region of highly conserved sequence homology, which results in a local high frequency of intertypic recombination.

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589 SUPPRESSION OF TEMPERATURE-SENSITIVE PHENOTYPE IN REOVIRUS: ALTERNATE PATHWAYS FROM ts MUTANT TO WILD PHENOTYPE, Robert F. Ramig, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, TX 77030

A large number of independently isolated, spontaneous revertants of temperature-sensitive (ts) mutants, representing all 7 reovirus type 3 mutant groups, were examined to determine whether they were intragenic revertants or contained extragenic suppressor mutations. Back-cross to wild type showed that 25 of 28 revertants contained the parental ts mutation and were, therefore, suppressed pseudorevertants in which the suppressor mutation and parental mutation were in genes separable by recombination (reassortment). In addition to the parental mutation, nonparental mutations were rescued from some of the suppressed pseudorevertants. A number of the nonparental ts lesions recombined with all of the previously defined mutant groups. Recombination analysis showed that these new mutations fell into three recombination groups that we have designated tsH, tsI and tsJ (1). Representative mutants of groups H, I and J were mapped into genome segments M1, L3 and S1, respectively. During the analysis of segment segregation in the progeny of the mapping crosses, it was noted that, in some of the recombinant progeny, ts genotype was masked by the presence of a suppressor mutation. Documentation of suppression in these recombinant progeny was necessary to firmly establish the location of the ts lesion, since the mapping strategy required that the genotype of the recombinant progeny be determined. A method for mapping the location of the suppressor mutation in a suppressed pseudorevertant has been developed. This method does not require that the suppressor mutation have an intrinsic ts phenotype. The results of such a mapping study will be presented. Possible mechanisms for the suppression of ts phenotype will be discussed.

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Genetic and Molecular Basis of Integration and Transformation

590 THE ORGANIZATION AND EXPRESSION OF VIRAL SEQUENCES INTEGRATED IN CELLS TRANSFORMED BY ADENOVIRUS 2 AND SV40, Joseph F. Sambrook, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; Michael Botchan, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; Shiu-Lok Hu, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; James Stringer, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

We have cloned the viral DNA together with its flanking host sequences from several lines of cells transformed by adenovirus 2 and SV40. From analyses of these clones we draw the following conclusions:

(i) the integrated viral sequences are not always colinear with those of the viral DNA. Deletion of viral sequences is common and at least in two cases inversions have been found within the integrated viral DNA.

(ii) integration occurs in different locations in both the cellular and viral sequences in different cell lines. There are no sequences common to any of the integration sites so far analyzed.

(iii) in some case amplification of viral sequences seems to occur after integration of adenovirus DNA into the genome of certain lines of transformed cells.

(iv) integration of viral DNA produces rearrangement of the flanking cellular sequences.

(v) in general, all early viral promoters present in the integrated genome are active, as are all terminators of early RNA synthesis.

591 POLYOMA VIRUS HR-T GENE PRODUCTS, Thomas Benjamin, Gordon Carmichael, Brian Schaffhausen, Department of Pathology, Harvard Medical School, Boston, MA 02115

A combination of DNA sequencing and analysis of T antigen forms of polyoma virus hr-t mutants has led to the conclusion that both the 22K small T antigen and the 56K middle T antigen are direct products of the hr-t viral gene (1,2). This gene is required for many if not all of the cellular changes accompanying transformation, including the acquisition of ability to grow in soft agar (3), changes of the cell surface (lectin agglutination), changes of the cytoskeleton (loss of 'actin cables'; 4), and morphological transformation (4). Hr-t mutants show DNA alterations within roughly a 325 base pair sequence in the proximal early region, a region known to code for identical protein sequences in the 22K and 56K T antigen proteins. The small T antigen appears to reside predominantly in the cytoplasm in a soluble form, while the middle T antigen is localized predominantly in the plasma membrane with small amounts seen in the nuclear fraction.

The role of the hr-t gene appears to be that of a pleiotropic regulatory gene whose major purpose is to alter the physiological state of its host to permit virus growth (5). Recently we have demonstrated a protein kinase activity in T antigen immune precipitates which may relate to the role of the hr-t gene in vivo. In the in vitro assay this activity leads to phosphorylation of the 56K protein itself; it is not seen in various hr-t mutants making truncated or slightly altered 56K proteins (6). This hr-t gene product may therefore be a protein kinase (carrying out autophosphorylation in the immune precipitates) or may be closely associated with a cellular kinase which fails to recognize the altered middle T antigen of hr-t mutants. Further attempts to characterize 56K and 22K proteins will be described.

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592 DO TERMINAL REDUNDANCIES IN THE PROVIRUS OF MOUSE MAMMARY TUMOR VIRUS MEDIATE REGULATION OF VIRAL GENE EXPRESSION BY GLUCOCORTICOID HORMONES?

Harold E. Varmus and John E. Majors, Dept. of Microbiology and Immunology, Univ. of California, San Francisco, 94143.

Proviruses of retroviruses are often found endogenous to normal cells and can be introduced into cells by experimental infection; in all studied examples, proviruses by be accommodated at any of several sites in host genomes, but the structure of the proviral DNA is generally similar. Proviruses are composed of viral genes arranged in the order found in the viral RNA genome and of direct terminal repeats of several hundred nucleotide pairs derived from sequences unique to both ends of viral RNA (for examples, see refs. 1-3). In this configuration, a complete copy of viral RNA is positioned "downstream in a transcriptional sense" from sequences present at the 3' end of viral RNA, suggesting the possibility that the "3 " sequences might promote and regulate transcription of viral genes.

We are attempting to assess this hypothesis using the mouse mammary tumor virus (MMTV), since transcription of MMTV DNA in a variety of infected cells is dramatically stimulated by glucocorticoid hormones (4,5). The direct terminal repeats in MMTV DNA are ca. 1200 base pairs in length. We have cloned the repeated region as well as surrounding DNA from unintegrated proviral MMTV DNA in order to determine its sequence and subject it to functional tests. The cloning in either plasmid or bacteriophage lambda vectors has been complicated by our inability to obtain clones containg complete copies of viral DNA; successful propagation of cloned DNA appears to depend upon the absence of a short sequence from the gag region of the viral genome To circumvent this difficulty, we have cloned the relevant portions of the viral DNA either by using Pst I fragments of unintegrated viral DNA (there by dissociating the redundancy from the unclonable sequences; ref. 6) or by using the provirus of a deletion mutant which lacks the unclonable sequence. In the latter case, we have obtained in a single cloned Eco RI fragment the entire truncated provirus plus several thousand bases of flanking cellular DNA; since this provirus is the only viral DNA in a clone of infected mink lung cells in which MMTV RNA synthesis is under glucocorticoid control the cloned DNA is very likely to contain the signal necessary for the hormonal response.

We will present a progress report on our efforts to sequence the interesting regions of these cloned DNA's and to determine their functional attributes.

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593 THE NATURE AND ORIGIN OF THE TRANSFORMING GENE OF AVIAN SARCOMA VIRUSES,

Hidesaburo Hanafusa, Lu-Hai Wang, Roger Karess, Teruko Hanafusa, William S. Hayward, Carlo Moscovici*, The Rockefeller University, New York, N.Y. 10021; *Tumor Virus Laboratory, V. A. Hospital, Gainesville, Fl. 32602

A class of new avian sarcoma viruses, named recovered sarcoma viruses(rASV), have been isolated from chicken or quail tumors produced by infection of transformation-defective (td) mutants of Rous sarcoma virus (RSV).[1,2,5] Since td mutants lack more than 70% of the src gene, the src gene of rASV must have been derived from cellular sarc sequences through genetic recombination.[2,3]

Most of the rASV isolates were non-defective and appeared to re-acquire the full src gene,[2,3] and its gene product, $pp60^{src}$, is fully expressed in transformed cells.[4] Fingerprint analysis of rASV showed that the src sequences of rASV are very similar to those of standard RSV, but not identical. Further, oligonucleotides specific to rASV obtained from chickens differed from those specific to quail-derived rASV.[5]

Td mutants are not always capable of generating rASV. Mutants which have a complete deletion of the src gene lack this capacity. Comparison of the RNA of various mutants seems to suggest that the retention of the sequence at the 3' end of the src gene in the td mutant is required for the generation of rASV. One td mutant, whose deletion is mainly within src gene, but extended into the 3' end of the env region, appears to consistently generate rASVs which are defective in env or in env and pol. This implies that the td virus-sequences required for the recombination with endogenous sarc are also located in the boundary of env-src genes and perhaps a similar sequences exist at the boundary of pol-env genes.

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594 THE TRANSFORMING GENE PRODUCT OF ROUS SARCOMA VIRUS IS A PROTEIN KINASE THAT PHOSPHORYLATES TYROSINE, Tony Hunter and Bartholomew Sefton, Tumor Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, California 92138.

The product of the src gene of Rous sarcoma virus (RSV) is a 60,000 dalton phosphoprotein, $pp60^{src}$, which has an associated protein kinase activity. It has been proposed by Collett and Erikson that RSV transforms cells through unscheduled phosphorylation of cellular proteins by $pp60^{src}$. The immunoglobulin heavy chain is phosphorylated by the protein kinase activity associated with $pp60^{src}$ in immunoprecipitates made with antitumor serum. We have examined the substrate specificity for this reaction and found that the acceptor amino acid is a tyrosine. Phosphorylation of tyrosine is an unprecedented protein modification, serine and threonine being the major phosphate acceptors in proteins. Despite the fact that a protein kinase phosphorylating tyrosine has not previously been reported, we have made several further observations which suggest that $pp60^{src}$ also phosphorylates tyrosine in vivo and that therefore the modification of proteins by phosphorylation of tyrosine is essential to the malignant transformation of cells by RSV.

We have found phosphotyrosine in proteins in a wide variety of uninfected cells. It is, however, an extremely rare modified amino acid, being present at about 1/3000th the level of phosphoserine. In cells transformed by RSV the level of phosphotyrosine in proteins rises about eight-fold. Cells infected by mutants of RSV temperature sensitive for transformation contain almost normal levels of phosphotyrosine at the restrictive temperature, but possess nearly wild type levels at the permissive temperature. The level of phosphotyrosine in mutant infected cells rises rapidly upon shift from the restrictive to the permissive temperature, being substantially increased within 1 hour. Elevated levels of phosphotyrosine are not found in all types of transformed cells, however, for example being low in polyoma transformed cells.

$pp60^{src}$ itself has two phosphorylation sites. The level of phosphorylation at the site in the C-terminal half is affected by mutations in the src gene and it has been suggested that this site undergoes autophosphorylation. Consistent with these notions we have now found that this site is a phosphotyrosine. This is the first demonstration of the presence of phosphotyrosine in a protein labeled in vivo. Phosphotyrosine is also present in a 50,000 dalton phosphoprotein which co-precipitates with $pp60^{src}$ from transformed chick cells. We infer that this protein is an intracellular substrate of $pp60^{src}$. It should clearly be possible to identify further substrates of $pp60^{src}$ on the basis of their containing phosphotyrosine.

$pp60^{src}$, the closely related cellular homologue of viral $pp60^{src}$, is present in all vertebrate cells. We have found that this normal cellular protein, obtained from both chicken and human cells, also phosphorylates tyrosine. This is additional evidence of the functional similarity of these structurally related proteins. It also demonstrates that all uninfected cells contain at least one protein kinase that phosphorylates tyrosine.

Genetic and Molecular Basis of Viral Regulation/Expression/Differentiation

595 HOW λ'S REPRESSOR AND CRO WORK, Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The right operator (O_R) in the chromosome of bacteriophage λ contains three contiguous sites that are recognized by the λ phage repressor. The operator overlaps, and controls divergent transcription from, the promoters P_R and P_{RM}. A variety of biochemical and genetic experiments have revealed how the operator controls the activity of these promoters. Among the salient points are the following:

1. λ repressor, which is required for lysogeny, functions without cofactors both as a positive and negative regulator of gene transcription.
2. Whether repressor has a positive or negative effect on transcription of adjacent genes depends in part on which of the three sites in O_R are occupied.
3. Cooperative interactions between DNA-bound repressors critically influence repressor's effect on gene expression.
4. Cro is a negative regulator required for lytic phage growth. Although cro has no obvious sequence similarity to repressor, it binds to the same three sites in O_R, apparently contacting many of the same functional groups on the DNA. It has a drastically different physiological effect, however, because it binds to these sites in a different order.

596 DNA-DEPENDENT TRANSCRIPTION OF MAMMALIAN GENES IN A SOLUBLE WHOLE-CELL EXTRACT, J.L. Manley, A. Fire, A. Cano, P. Sharp and M.L. Gefter, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

We have recently developed a new cell-free system to study the expression of eukaryotic genes. Reaction mixtures contain a dialyzed and concentrated HeLa cell extract, small molecules and cofactors required for transcription, and exogenously added DNA. When a cloned 2.2 kb restriction fragment of adenovirus 2 (Ad2) DNA which contains the major late promoter is added to the system, we detect large amounts of a 1.7 kb transcript. If another cloned 2.2 kb Ad2 restriction fragment, which does not contain a promoter, is added instead, no transcription occurs. We have shown that the 1.7 kb transcript initiates at the major late promoter by several methods, including fingerprint analysis of the 5' end. Total Ad2 DNA also functions efficiently as a template and in vitro transcription initiated at the major late promoter continues for a distance of at least 4.5 kb. We have also tentatively identified transcripts originating from early Ad2 promoters, using cloned DNA restriction fragments, as well as total Ad2 DNA, as templates. Finally, a cloned 8.0 kb restriction fragment of human DNA which contains the β-globin gene (kindly provided by T. Maniatis and C. O'Connell) functions efficiently as a template for in vitro transcription. Transcription initiates at, or near, the mRNA cap site and continues at least 1400 nucleotides. The β-globin promoter functions in vitro approximately 30% as efficiently as does the Ad2 major late promoter.

597 TUMOR VIRUS INTEGRATION AND EXPRESSION DURING EMBRYOGENESIS AND DIFFERENTIATION, Rudolf Jaenisch and Detlev Jähner, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany

A subline of mice (= BALB/Mo) carrying the exogenous Moloney leukemia virus (= M-MuLV) in its germ line has been derived previously by infecting preimplantation mouse embryos with virus. Using a similar approach, new mouse lines have been obtained which transmit the M-MuLV genome genetically. One male derived from an infected embryo transmitted the virus at four independently segregating loci which were characterized after digestion with EcoRI by Southern blotting analysis. The four different genotypes led to two distinct phenotypes in the mice. Viremia and fast developing leukemia were associated with the presence of an M-MuLV-specific band of 6.5 x 10^6 and 11 x 10^6 dalton, respectively. Non-viremic animals transmitted the M-MuLV genome as a 5.7 x 10^6 or a 13 x 10^6 dalton band. Tissue-specific expression of M-MuLV was analyzed in viremic animals carrying the M-MuLV genome as an 11 x 10^6 dalton band. In addition to spleen and thymus, testes, kidneys, heart and lungs expressed viral specific RNA sequences. In contrast, animals transmitting the M-MuLV genome as a 13 x 10^6 dalton band did not express viral specific RNA sequences in any organ tested. Occasionally, however, virus became activated in these mice leading to M-MuLV-specific viremia. BALB/Mo mice, on the other hand, carrying the M-MuLV genome as a 17 x 10^6 dalton EcoRI fragment in their germ line express M-MuLV-specific RNA sequences in spleen and thymus only. These results indicate that leukemia viruses can integrate into the mouse germ line at different chromosomal locations. They strongly suggest that tissue-specific expression of the viral genome is influenced by the site of chromosomal integration.

In a second line of experiments embryos have been microinjected with virus at day 8 and 9 of gestation. This is a stage of embryonal development when organogenesis begins. Viremic mice obtained from these embryos had viral specific DNA and RNA sequences in every organ. These results suggest that the most important parameter determining the tissue tropism of infection with M-MuLV is the stage of differentiation at which encounter with virus takes place.

598 MUTANTS OF AVIAN ERYTHROBLASTOSIS AND MYELOCYTOMATOSIS VIRUSES WITH ALTERED TRANSFORMATION SPECIFICITY, Thomas Graf & Hartmut Beug, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg; Dominique Stéhelin, Institut Pasteur de Lille, 15 rue C.Guérin, Lille, France; Gary Ramsay & Michael J. Hayman, Imperial Cancer Research Fund Labs., Lincoln's Inn Fields, P.O. Box 123, London WC2A 3PX, England.

Avian erythroblastosis (AEV) and avian myelocytomatosis virus strain 29 (MC29) have previously been shown to transform hematopoietic cells as well as fibroblasts in vitro even after clone purification of the respective viruses (1,2). Hematopoietic cells transformed by AEV have the properties of erythroblasts whereas MC29-transformed hematopoietic cells resemble macrophages (3). Cloned fibroblasts transformed by these strains can be distinguished by the pattern of transformation parameters they express (4). The different biological properties of these two virus strains have been attributed to two new transformation specific sequences designated erb for AEV and mac for MC29 (5,3).
We have now been able to isolate a mutant of AEV (td359 AEV) (5) and 3 mutants of MC29 (td4A MC29, td10C MC29 and td10H MC29) which have lost the ability to transform hematopoetic cells but which have retained the ability to transform fibroblasts. These mutants show an increase in the mobility on SDS-PAGE of their presumptive transforming gag-erb fusion protein p75 AEV (7) and in the gag-mac fusion protein p110 MC29 (8), respectively. In addition, tryptic peptide analysis of these proteins revealed that they lack some of the transformation specific sequences and that they acquired in some cases additional gag-specific sequences.

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Animal Virus Vectors for Transducing Mammalian Cells

599 TRANSDUCING VECTORS FOR MAMMALIAN CELLS, Paul Berg, Department of Biochemistry, Stanford Univ. Sch. of Medicine, Stanford, California 94305

Recent advances in molecular cloning methodologies have provided promising opportunities to study the structure and organization of mammalian genes. Our aim has been to develop a comparably simple approach for analyzing the expression and regulation of the isolated genes, not in bacterial or yeast hosts under the control of bacterial and yeast genetic signals, but in the natural context of mammalian genomes - the mammalian cell nucleus. To achieve this we have adopted the SV40 chromosome - a single, covalently circular, duplex DNA molecule of 5243 base pairs, which can infect, multiply and be expressed in monkey cells - as a vector for transducing exogenous genes into mammalian cells. In outline, recombinants are constructed in vitro by joining segments of wild type, mutant or otherwise substituted or rearranged SV40 DNA to suitably modified segments of exogenous DNAs; the recombinant genomes are propagated and cloned as virions by complementation with appropriate SV40 ts mutants.

In this protocol, recombinant genomes must contain the origin of viral DNA replication and at least one functional gene-early or late - to complement the ts defect of the helper, which in turn provides the virus function lacking in the vector genome. Moreover, for recombinant genomes to be propagated as virions they can not be larger than 5.24 kb, the size of wild type DNA.

We have already reported (1) the construction of recombinants in which a rabbit β globin cDNA segment was substituted for the protein coding region of a substantial part of SV40's VP1 gene. Infection of CV-1 cells with this recombinant yielded the expected spliced but substituted mRNAs and rabbit β globin protein (1). The same β globin cDNA segment has also been inserted into SV40 DNA in place of specific segments of the late and early regions. Infections with these recombinants yields substantial amounts of β globin only when the transcript containing the β globin cDNA sequence can be spliced and the β globin initiator AUG codon is properly oriented relative to the capped 5'-end and/or the leader. DNA segments coding for E. coli guanine phosphoribosyl transferase (gpt), mouse dihydrofolate reductase (DHFR) and Drosophila melanogaster histone H2b (H2b) have also been introduced into early and/or late SV40 vectors with analogous results. After infection the two enzymes and histone H2b protein are made provided the coding sequence is located at a site that permits it to be transcribed and processed to a mature mRNA. The lecture will describe these experiments in detail and discuss more recent progress in the construction of plasmid-type vectors for propagating genes in cultured cells.

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Host Genetics

600 GENETIC CONTROL OF MuLV EXPRESSION AND OF SPONTANEOUS LYMPHOMA IN CROSSES OF HIGH- AND LOW-LYMPHOMA MOUSE STRAINS. Frank Lilly, Suzie Chen and Maria L. Duran-Reynals, Albert Einstein College of Medicine, Bronx, New York 10461

Genetic control of MuLV expression and of spontaneous lymphoma in crosses of high- and low-lymphoma mouse strains. Nearly all mice of the inbred AKR strain develop lymphoma before one year of age, but F_1 mice from crosses of AKR with mice of various low-lymphoma strains develop the disease only at low incidences and/or much later in life in the large majority of such crosses that have been studied. It appears that one or more dominant gene transmitted from the low-lymphoma parental strain can suppress the development of the disease.

AKR mice express high levels of ecotropic murine leukemia virus (MuLV) from birth or soon thereafter, and MuLV with xenotropic and polytropic host ranges are usually detectable in their thymuses at six to eight months of age. (Gross MuLV Passage A is a strain of leukemogenic virus originally isolated from lymphomatous AKR tissues, but the relation between the relatively well characterized MuLVs present in AKR tissues and the lymphomagenic agent in Gross MuLV preparations remains to be clarified.)

This laboratory has previously reported that genetic analysis of two such crosses, AKR x BALB/c and AKR x RF, indicated that alleles at the *Fv-1* locus transmitted from the low-lymphoma parent were major factors for lymphoma suppression. In each case, the *Fv-1* resistance allele concommitantly suppressed some aspect of MuLV expression. We now report results from three more such crosses: AKR x DBA/1, AKR x C57L and C58 x DBA/2. Mice of segregating generations of these crosses were examined for their phenotypes at the *Gpd-1* locus (closely linked to *Fv-1*) and observed for occurrence of lymphoma. In none of the three crosses was there an association between *Gpd-1* type and the disease, indicating that genes other than *Fv-1* suppress lymphoma in these cases. Moreover, no significant association between lymphoma and endogenous MuLV expression was detected in two of the three crosses, suggesting that the lymphoma-suppressing genes operate by affecting some lymphoma-associated factor other than endogenous MuLV. In only one of the three crosses was an association noted between lymphoma and *H-2* type, although the *H-2* complex has often proved to be a significant determinant of lymphoma susceptibility in other crosses.

601 FRIEND ERYTHROLEUKEMIA ANTIGEN: A VIRUS-INDUCED, DIFFERENTIATION-RELATED CELL SURFACE STRUCTURE, Rex Risser, Patricia Jelen and Carol Sinalko, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

Serologic studies of primary Friend erythroleukemia cells indicated that these cells express on their surfaces a determinant, FE antigen, which is induced by spleen focus-forming virus (SFFV). This antigen cross-reacts with an antigen found on cells of the hematopoietic organs of uninfected mice, i.e. fetal liver, bone marrow and spleen. Not all mouse strains express the same quantities of this antigen; thus far it has only been detected on bone marrow of those strains which carry the dominant Fv-2^s virus sensitivity allele and are therefore susceptible to SFFV-induced erythroleukemia. In view of Axelrad's recent proposal that the Fv-2 locus controls virus sensitivity by controlling the rate of cycling of erythroid precursor cells, the existence of an antigen shared by SFFV-infected cells and bone marrow from Fv-2^s mice may reflect similar mechanisms involved in both proliferative states.

An unusual feature of FE antigen is its sensitivity to erythropoietin, but not other inducers such as DMSO, or thymopoietin. Treatment of Friend spleen cells with 1 unit/ml of erythropoietin for 15-30 min at 37° leads to the loss of FE antigen as detected in serologic tests, though no change occurs in the expression of the other virus-induced FMR cell-surface antigen(s). Preliminary results of experiments designed to investigate the effect of erthropoietic factors on SFFV-infected cells will be presented.

602 DNA - Mediated Gene Transfer: Theory and Application, Michael Wigler, Manolo Perucho, Leah Lipsich, David Lane, Dan Klessig, Terry Grodzicker, Michael Botchan and Doug Hanahan, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

Certain cultured cell lines can readily be transformed by exogenous DNA when given as a calcium phosphate coprecipitate. Although stable transformants, detected by biochemical selection, can arise from exposure of 10^6 cells to as little as 50 pg of a purified gene. such efficient gene transfer requires the use of vast excesses of carrier DNA. Cells which incorporate a selectable marker, such as thymidine kinase, are also likely to incorporate significant amounts of carrier DNA, a process we have called cotransformation. By adding well defined DNA sequences to the carrier it is possible to construct cell lines containing virtually any defined DNA sequence. Genetic and biochemical data indicate that selectable markers and carrier DNA become covalently linked in the transformed cell and are cointegrated into a structure we have called the pekelasome. We do not know at present whether pekelasomes are sometimes or even usually integrated into host chromosomes.

Cotransformation has been used to study the expression of unselected transforming elements of viral origin. The 3.4 kb Bam H-1 fragment of Herpes Simplex Virus-1 coding for thymidine kinase (HSV-1 tk) is expressed constitutively when cotransfered using the adenosine phosphoribosyl transferase gene as the selective marker, suggesting this HSV-1 Bam fragment carries its own promoter. We have also studied the expression of the early regions of both SV40 and adenovirus in cotransformants. In contrast to the HSV-1 tk, the mere presence of these early viral regions in transformants does not insure their expression. Moreover, the expression of these genes is often phenotypically unstable.

DNA-mediated transformation can be used to isolate cellular genes coding for selectable markers. Ligation of restriction cleaved genomic DNA to bacterial plasmid pBR322 prior to transformation of animal cells results in a transformed host containing pBR322 sequences adjoining the selected marker. The pBR322 can then be used as a transducing element to rescue the selected marker into E.Coli. In this way we have isolated a 1.4 kb chicken gene coding for thymidine kinase. The generality of this method for gene isolation will be discussed.

603 MOLECULAR CLONING OF A TRANSFORMING ALLELE OF CHEMICALLY-TRANSFORMED MOUSE CELLS, R.A. Weinberg, B. Shilo, M. Merrick, C. Shih, L.C. Pahdy, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

DNA extracted from several mouse clones transformed by chemical carcinogens has been applied to mouse fibroblast NIH3T3 cultures, using the transfection technique of Graham and Van der Eb. Foci of transformed cells have been derived from such transfections. The transformants are able to grow in soft agar, and are tumorigenic in newborn mice. These results show that the phenotype of transformation can be transferred by naked DNA. They also indicate that in those cases the transforming allele is dominant.

Treatment of one of the transforming DNAs (extracted from a C3H10T1/2 cell line transformed by 3-methylcholanthrene) with a series of restriction enzymes showed that BamHI does not inactivate its biological activity. Using a cloning strategy developed in yeast (1), we have cloned a DNA fragment having sequence homology with the gene carrying the transforming allele. A homologous gene can be demonstrated to exist as a single copy in normal mouse cells, using the Southern blotting technique. In cells which have been transformed via transfection, an additional copy of a homologous sequence is shown to be acquired. The cloned sequence has no apparent homology with the sarc regions of Moloney murine sarcoma virus and Avian sarcoma virus.

The potential use of the cloned DNA fragment will be described. We will determine whether cells transformed by independent carcinogenic events, always transfer the same transforming gene during DNA transfections. The results would indicate whether a single or multiple normal cellular genes are potential targets for the carcinogenic events. In order to study the effect of the carcinogen on the gene, differences between the transforming allele and its counterpart sequence in normal cells will be looked at. Finally the transcription of the gene will be monitored in normal and transformed cells, to ask whether the carcinogen is affecting the promoter of the gene.

1. Hicks, J.B., Hinnen, A. and Fink, G.R. 1978. Cold Spring Harbor Symp. Quant. Biol. 43: 1305-1313.

Genetics of Virulence and Persistence

604 THE ROLE OF THE REOVIRUS HEMAGGLUTININ IN VIRAL VIRULENCE, Bernard N. Fields, Howard L. Weiner, Dennis A. Drayna, and Arlene H. Sharpe, Harvard Medical School, Boston, Mass. 02115

Recombinants derived from reoviruses type 1, 2 and 3 have been used to study virus-host interactions. We have found that the S1 dsRNA segment, the segment that encodes the σ1 polypeptide, encodes the viral hemagglutinin. Thus the σ1 polypeptide is the hemagglutinin. The hemagglutinin is responsible for specificity in humoral and cellular immunity. It also determines cell tropism in the nervous system, binding to cellular microtubules, and shut-off of host cell DNA synthesis. The specific interaction of the hemagglutinin with the host thus determines several facets of viral host interactions and virulence.

605 HOST GENE PRODUCTS AND VIRUS INFECTION, Michael B. A. Oldstone, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Although a great deal of information has accumulated about viral penetration and replication in many types of host cells, comparatively little is known about the initial binding of virus to cells or about specific receptors for viruses. The product of the major histocompatibility gene complex (MHC) is of biological interest because of its fundamental role in certain cell-cell membrane recognition systems, its control of important gene products, and its association with susceptibility to some diseases. For these reasons we determined whether the MHC provides specific receptors for a variety of viruses. Our approach was to use a library of cells with and without MHC determinants and determine the extent to which each culture supported the penetration, expression and release of infectious viruses. Murine F9 and PCC4 teratoma cells do not express H-2 major transplantation antigens according to virus specific T lymphocyte cytotoxic or serologic assays. However, such cells can be easily infected with and rapidly replicate many types of viruses including Coxsackie B3, mouse hepatitis, Sindbis, Semliki Forest, lymphocytic choriomeningitis, Pichinde, vesicular stomatitis, herpes simplex type 1 and herpes simplex type 2 to the same extent as do murine F12 teratoma cells and mouse embryo fibroblasts, all of which express the H-2 determinants. In contrast, F9 and PCC4 cells are not productively infected with murine cytomegalovirus, whereas F12 and mouse embryo fibroblast cells are. The undifferentiated pleuripotential PCC4 cell line could be induced to differentiate in vitro by treatment with N,N-dimethylacetamide. Upon differentiation, PCC4 cells were fully permissive to MCMV. Further, undifferentiated PCC4 cells which did not express viral antigen or release infectious virus following infection with MCMV upon differentiation now express viral antigens and release infectious virus. Murine CMV DNA was detected in undifferentiated cells but was amplified 4 to 5-fold (4 copies/cell) in differentiated cells. These results indicate that a cell's possession of H-2 antigens is not a requirement for infection with Coxsackie B3, mouse hepatitis, Sindbis, Semliki Forest, lymphocytic choriomeningitis, Pichinde, VSV, HSV or MCMV infection and that MHC products are not specific receptors for these viruses. This work was done in collaboration with Dr. F. Dutko.

606 VIRAL MUTATION IN PERSISTENT INFECTION. John J. Holland, Katherine R. Spindler, Elizabeth A. Grabau, Bert L. Semler, Charlotte L. Jones, Frank M. Horodyski, and David Rowlands, Department of Biology, University of California, San Diego, La Jolla, California 92093.

More than six years ago we established persistent infection of BHK_{21} cells with a mixture of cloned VSV Indiana infectious virus and highly purified defective interfering (DI) particles. Since that time nearly 100% of these cells have expressed viral antigens and resisted homologous challenge, yet they shed only very low levels of mature infectious virus and mature DI particles while the cells grow at normal or near-normal rates. The shed DI particle types compete unpredictably, and change continuously with time. The phenotype of the shed infectious virus has generally been temperature-sensitive and small plaque, and slowly-replicating at all temperatures. Oligonucleotide maps of RNA of the infectious virus shed at various times by carrier cells showed that the viral genome is undergoing massive and continuous mutation during years of persistent infection[1]. Likewise, peptide mapping shows that not all of these mutations are silent third base mutations, since many peptide map changes are evident, particularly in the N,G, and M proteins. RNA sequencing studies of shed virus genome RNA termini shows mutational change in both the 5' and 3' ends. Thus, it appears that the entire genome, in both coding and non-coding regions, is undergoing a remarkable degree of mutational change during persistent infection, and yet the recovered mutants show considerable genetic stability. True revertents cannot be obtained by lytic passage of recovered virus, although several oligonucleotide map changes were obtained after more than 100 lytic passages of cloned virus recovered after five years of persistent infection.

The virus recovered after more than five and one half years of persistent infection is very slow-growing and upon initial isolation gives very poor yields of infectious virus from lytically infected cells. Also, this recovered virus is now able for the first time to establish (after initial cytopathology) persistent infection of BHK_{21} cells without addition of DI particles. For the first five years of persistence all cloned recovered virus killed 100% of cells in the absence of DI particles. Recovered virus also exhibits altered and variable *in vivo* behavior.

We are currently investigating the possible role of DI particles in the evolution of massive genome mutations of infectious standard virus. Preliminary evidence suggests that they do influence the evolution of the standard virus genome in persistently infected cells.

[1]Holland, J.J., Grabau, E.A., Jones, C.L., and Semler, B.L. (1979). Cell 16: 495-504.

607 ANTIGENIC VARIATION OF VISNA VIRUS. J.E. Clements*, N. D'Antonio*, O. Narayan*, F.S. Pedersen°, W.S. Haseltine°. *Johns Hopkins School of Medicine, Baltimore, Maryland 21205. °Harvard Medical School, Boston, Massachusetts 02115.

Visna virus, an exogenous retrovirus of sheep causes a slowly progressive degenerative disease of the central nervous system. Despite the hosts humoral and cell mediated immune response, the virus persists in the animal. Viruses isolated from a persistently infected sheep early after infection were neutralized by early immune sera from this animal to the same extent as the parental virus (1). Those isolated from 1 year on were found to be parental type as well as antigenically distinct viruses. The sheep subsequently developed neutralizing antibody to these variant viruses. These antigenic variants are genetically stable and are fully virulent in sheep. Antigenic variation of visna virus in sheep appears to be a continuous process of newly emerging viruses and may be responsible for the progressive nature of the disease. To investigate the genetic basis of antigenic variation we have studied seven variants isolated from a persistently infected animal.

Antigenic variants of visna virus have been compared using the genomic RNA and analyzing the large RNase T_1-resistant oligonucleotides. Mutants isolated from a persistently infected sheep contained a small number of changes in their oligonucleotide patterns when compared with parental virus. To determine whether the changes in the nucleotide structure were clustered in one region of the genome, the oligonucleotides of the parental and a mutant RNA were ordered along the genome with respect to the 3' polyadenylated end. All but one difference between the parental strain and the antigenic mutant used for mapping were located within 2 kilobases from the 3' terminus. The electrophoretic mobilities of some of the oligonucleotides which differed from the parental to the mutant suggest that they might be derived by simple mutation. To determine whether this was how the mutants arose the complete nucleotide sequence of several oligonucleotides were determined. These analyses showed that a number of the oligonucleotides which were different between the parental and mutant could be accounted for by single base changes. Thus, based on these studies we propose that multiple mutational events in the region of the genome which codes for the viral antigen that elicits neutralizing antibody permit the virus to escape the immune response and may, in addition, be responsible for the slow progressive nature of the disease.

(1) Narayan, O., Griffin, D.E. and Clements, J.E. J. Gen. Virol. 41: 343-352, (1978).

608 PATHOGENESIS AND PERSISTENCE OF PARAMYXOVIRUSES, P. W. Choppin, C. D. Richardson, D. C. Merz, W. W. Hall, and A. Scheid. The Rockefeller University, New York, N.Y.

The biological roles of paramyxovirus envelope proteins (HN, F, and M) have been studied. F is involved in virus penetration, cell fusion and hemolysis, and is activated by cleavage by a host protease into 2 disulfide-bonded subunits (F_1 and F_2). The ability of the virus to undergo multiple cycle replication, spread, and cause disease is dependent on the presence of an activating protease in the host; thus cleavage of F is a major determinant of pathogenesis. The N-terminus generated on F_1 by cleavage is involved in its biological activity. The amino acid sequence in this region is hydrophobic and similar in Sendai, SV5 and NDV. In an attempt to design specific inhibitors of virus replication, cell fusion and hemolysis, oligopeptides analogous to this region of F_1 were synthesized and found to be specific and highly-active. Structure-activity studies showed that amino acid sequence, peptide length, and N-terminal carbobenzoxy group, C-terminal esterification, and steric configuration of amino acids affect inhibitory activity. Specific inhibition of influenza virus has also been obtained with peptides which resemble the N-terminal region of HA_2. These results have provided information on the mechanism of action of viral proteins in penetration and membrane fusion, and have also suggested a new approach to specific inhibition of viral replication.

Studies with mono-specific antibodies have shown that antibodies to HN can inhibit dissemination of infection by released virus, but not spread from cell to cell via membrane fusion, whereas antibodies to F completely inhibit spread of infection. Thus to be effective a paramyxovirus vaccine must stimulate antibodies to F. These results, coupled with findings by Norrby and co-workers that formalin-inactivated paramyxovirus vaccines did not elicit anti-hemolyzing antibodies, provide an explanation for vaccine failure, and for atypical and severe diseases, e.g., atypical measles, that occurred in individuals who received inactivated vaccines and were later infected. In the absence of F antibodies, infection could spread at the same time that there was a secondary immune response to HN and other viral proteins, setting the stage for a pathological immune reaction.

Patients with subacute sclerosing panencephalitis (SSPE), caused by persistent measles virus infection, lack antibodies to M protein despite high serum and cerebrospinal fluid (CSF) titers of antibodies to other measles proteins, suggesting a host-dependent lack of M synthesis in the brain. Evidence supporting this has been obtained in cultures of SSPE brain cells which synthesized other measles proteins but not M. We also found that a patient with severe mental retardation and epilepsy 24 years after measles encephalitis had high titers of serum and CSF antibodies to all measles proteins except M, suggesting that: 1) virus persisted in the brain; 2) chronic neurological disease caused by measles virus is not limited to SSPE; 3) persistent measles infection may cause some cases of mental retardation and epilepsy.

Genetics of Polymorphism and Evolution

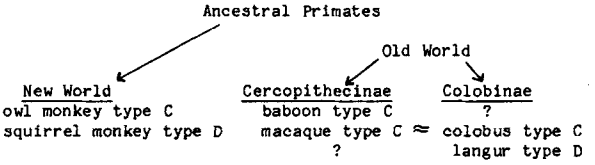
609 EVOLUTION OF TEMPERATE BACTERIOPHAGE, David Botstein, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Molecular studies of the genomes of several families of bacteriophage (particularly the group which the temperate coliphage lambda and the temperate Salmonella phage P22) suggest that such families evolve as a group of interbreeding interchangeable modular elements. Natural selection, in this view, acts upon the efficiency with which each module does its job and upon retention of easy interchangeability without loss of function and proper regulation. Thus the product of evolution is not a given bacteriophage, but the group of interbreeding interchangeable modular parts. Each virus found in nature is seen as a sample of a large variety of possible modular assemblies which work well in a given ecological niche.

The evidence for this view of bacteriophage evolution will be summarized, and the possible applicability of this idea to animal virues will be discussed.

610 ORIGINS AND DISTRIBUTION OF GENETICALLY TRANSMITTED RETROVIRUSES OF PRIMATES, George J. Todaro, Laboratory of Viral Carcinogenesis, National Institutes of Health, Bethesda, Maryland 20205

Six different endogenous retroviruses of primates have been isolated during the past few years. Two are from New World primates - a type C virus from owl monkeys and a type D virus from squirrel monkeys. Four are from Old World primates - type C's from baboons, macaques and colobus monkeys and type D's from langur monkeys. The colobus type C virus came from a kidney cell culture, and was isolated on an unusually permissive human carcinoma line, A549. 50-100 copies of viral related information are found in colobus cellular DNA with related sequences in related primate DNAs. The colobus viral RNA and proteins are related to macaque type C viruses and not to baboon viruses. The isolates are summarized as follows:



All have genomes that are represented in multiple copies (20-200 per haploid genome) in normal cellular DNA of the species of origin. Two of these are readily isolated from most normal tissues; high frequency activation occurs with baboon type C and squirrel monkey type D viruses. The others represent rare isolates that result from prolonged cocultivations and that replicate only in a restricted number of host cells. An example of naturally occurring transmission between primates is the MP-MV virus, commonly isolated from rhesus monkeys, but that hybridizes to a considerately greater extent to normal langur monkey DNA and has, presumably, been derived from endogenous langur type D virogenes. Other examples are RD-114, endogenous in cats that was derived from African monkey virogenes, and the gibbon-woolly monkey type C group that are most closely related to certain endogenous rodent viruses. Primates, thus, appear to have as much naturally transmitted retrovirus information as do rodents or any other mammalian orders.

611 ENVELOPE GENES AND c REGIONS IN NON-ACUTE AVIAL LEUKOSIS VIRUS ASSOCIATED DISEASE, Harriet L. Robinson[+], Philip N. Tsichlis[*] and John M. Coffin[†]. [+]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, [*]Department of Medicine, [†]Department of Microbiology and Molecular Biology, Tufts University School of Medicine, Boston, MA 02111

Avian leukosis viruses (ALVs) of endogenous origin do not appear to cause disease[1]. These viruses have similar genetic compositions to ALVs of exogenous origin which cause non-acute disease. The major genetic differences between endogenous and exogenous ALVs are found in their envelope genes and c regions. Envelope genes of endogenous origin code for subgroup E antigens ($\underline{env}^E$) whereas envelope genes of exogenous origin code for subgroups A, B, C, or D antigens ($\underline{env}^A$, etc.). Constant regions of endogenous viruses, $\underline{c}^n$, have characteristic oligonucleotides which are distinct from those in the constant regions of exogenous viruses, $\underline{c}^x$ [2].

We are using endogenous viruses and recombinants of endogenous and exogenous viruses to test for the role of envelope antigens and c regions in disease. Four cloned $\underline{env}^E$ $\underline{c}^x$ viruses (RAV-60s) have been compared for their oncogenic activity with a $\underline{env}^A$ $\underline{c}^x$ virus (RAV-1). These experiments demonstrated that envelope antigens are not critical for disease and that cloned isolates of ALVs cause more than one disease[3].

A cloned $\underline{env}^E$ $\underline{c}^x$ virus (NTRE-7) and a $\underline{env}^E$ $\underline{c}^n$ virus (RAV-0) are currently being tested for oncogenesis. NTRE-7 was chosen for this study since only the 3' portion of its c region has oligonucleotides characteristic of $\underline{c}^x$ [4]. The results to date of these tests suggest that $\underline{c}^x$ is the oncogenic agent in non-acute disease.

How might $\underline{c}^x$ cause multiple independent diseases which occur as rare events? We suggest that (1) c regions contain promotors for viral RNA synthesis, (2) $\underline{c}^x$ contains a more efficient promotor than $\underline{c}^n$ and (3) non-acute disease follows the formation of critical viral-cell recombinants in which $\underline{c}^x$ promotes the expression of abnormally high and therefore oncogenic levels of cellular genes.

1) Motta et al. (1975). JNCI 55:685.
Crittenden et al. (1979). Avian Disease 23:646.
2) Coffin et al. (1978). J. Virol. 28:972.
3) Robinson et al. (1979). CSHSQB Vol. XLIV, in press.
4) Tsichlis et al. (1979). J. Virol, in press.

Paramyxoviruses/Rhabdoviruses

612 GROUP-SPECIFIC AND SUBGROUP-SPECIFIC NUCLEOTIDE SEQUENCES AS A BASIS FOR RNA TUMOR VIRUS CLASSIFICATION. Peter H. Duesberg, Dept. of Molecular Biology, University of California, Berkeley, California 94720

Virus classification, says Lwoff (1962), implies a hierarchy of characters, and a hierarchy implies a choice. As with other viruses, structural characters, such as the internal group-specific antigens coded by the gag gene, and the subgroup-specific envelope glycoproteins coded by env, have been chosen to classify RNA tumor viruses (Tooze, 1973). However, these classifications fail to accommodate many recently-identified transforming and nontransforming viruses which are defective in all three essential virion genes: gag, pol, and env. It is proposed here that all defective and nondefective RNA tumor viruses can be consistently classified, based on conserved terminal group-specific sequences, including the 3' terminal C-region, the 5' terminal sequences complementary to strong-stop cDNA, and conserved sequence elements of the gag gene. Specific sequences, which are unrelated to essential virion genes or altered virion genes, often correlate with oncogenic properties (Duesberg, 1980; Bister and Duesberg, 1980). The presence of such sequences in defective and nondefective oncogenic viruses and the lack of such sequences in nondefective leukemia viruses would be a basis to subclassify all defective and nondefective viruses of a given taxonomic group. Unlike other systems (Tooze, 1973), this subclassification is directly relevant to the oncogenic properties of these viruses. The system proposed will be discussed using viruses of the avian and murine tumor virus groups as models. LWOFF et al. (1962) CSH, 27: 51. Tooze (1973) The Molecular Biology of Tumor Viruses, CSH. Duesberg (1980) CSH, 44, in press; Bister and Duesberg (1980) ibid.

613 ROLE OF THE c REGION IN GROWTH RATES OF ENDOGENOUS AND EXOGENOUS AVIAN ONCOVIRUSES, John M. Coffin*, Philip N. Tsichlis* and Harriet L. Robinson†, *Tufts University School of Medicine, Boston, MA 02111 and †Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Endogenous and exogenous avian tumor viruses are closely related but differ in that endogenous viruses replicate more poorly in cell culture and do not cause leukemia or other disease in susceptible birds. To determine the regions of the genome responsible for these differences, several sets of recombinants between the endogenous virus RAV-0 and different types of exogenous viruses were prepared and selected for the host range of RAV-0 and either transformation or growth rate of the exogenous parent. The pattern of inheritance of various portions of the genome from either parent was determined by fingerprinting. We found that the only region of the exogenous virus genome which segregated with growth rate was the c region of several hundred nucleotides adjacent to the 3' end. A recombinant, NTRE-7, which contained the entire RAV-0 genome except for the c region, had a growth rate like that of exogegenous virus. The c region is closely related in sequence in all exogenous viruses but has a substantially different sequence in endogenous viruses. On the basis of these experiments, we define two alleles of c: c^x in exogenous virus which contributes to relatively rapid growth compared to c^n in endogenous virus. Our results are consistent with the hypothesis (of others) that the c region contains the promoter for viral RNA synthesis, and that the endogenous virus promoter is less efficient than that of exogenous virus. A possible role of the c region in leukemogenesis suggested by these results is being tested with NTRE-7.

614 THE NUCLEOTIDE SEQUENCE OF ROUS SARCOMA VIRUS, Dennis E. Schwartz, Richard J. Tizard, and Walter Gilbert, Harvard University, Cambridge, MA 02138

We are determining the nucleotide sequence of Rous sarcoma virus by the following method: (1) The viral 35S RNA is copied completely with reverse transcriptase using short calf thymus DNA oligonucleotides to prime synthesis at multiple points. (2) The resulting cDNA is fractionated and all fragments having a length greater than 300 nucleotides are isolated and cut with a restriction endonuclease capable of digesting single-stranded DNA. (3) After 5' or 3' terminal labeling, the fragments are isolated by a new two-dimensional polyacrylamide gel system and then sequenced directly by the chemical cleavage procedure. This new approach is simple and allows the sequence of any single-stranded RNA virus to be determined rapidly without the use of molecular cloning techniques.

615 RELATIONSHIPS AMONG TYPE C RETROVIRUSES BASED ON 5' TERMINAL NUCLEOTIDE SEQUENCES, Gerald G. Lovinger and Gerald Schochetman, Frederick Cancer Research Center, Frederick, MD 21701

The 5' terminal regions of six mammalian type C retroviruses (R-MuLV, GaLV-H, RD114, BaEV(M7), MMC-1, and MAC-1) have been sequenced. The chain lengths of these regions range from 126-144 nucleotides between the tRNA primer site and the 5' terminal cap structure. Sequences within this region are highly conserved among the retroviruses and show striking similarities to conserved 5' and 3' noncoding regions of eucaryotic mRNA. These sequences include (1) an AT rich hexanucleotide either analogous to a sequence observed in the 3' noncoding region of many mRNAs, or analogous to a putative RNA polymerase binding site, (2) a 10-14 nucleotide long palindromic sequence, and (3) a GUG sequence which has been proposed as a ribosome binding site. This region of the mammalian retroviruses, however, does not contain AUG initiation codons, indicating its lack of protein coding capacity. On the basis of the ALIGN computer program sequence comparisons, 3 equally distant mammalian groups of C type retroviruses were established - R-MuLV/GaLV-H, RDIII/BaEV, MMC-1/MAC-1. Members of the leukosis-sarcoma group, AMV and ASV, form a fourth type C group, distantly related to the mammalian groups. Thus, intergroup relationships between distantly related viruses not detectable by hybridization techniques are elucidated by sequence analysis. Based on these new relationships, an evolutionary scheme is proposed.

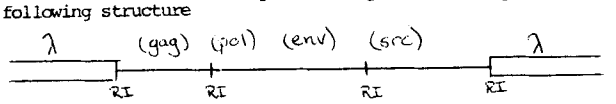
* R-MuLV - Rauscher murine leukemia virus; GaLV - gibbon ape leukemia virus; RD114 - endogenous cat virus; BaEV(M7) - baboon endogenous virus; MMC-1 - endogenous rhesus monkey virus; MAC-1 - endogenous stumptailed monkey virus.

616 CLONING AND CHARACTERIZATION OF "STRONG STOP" AND ADJACENT SEQUENCES OF SIMIAN SARCOMA VIRUS, Martin L. Scott, Henry S. Kaplan, and Kirk Fry, Stanford University School of Medicine, Stanford, Ca. 94305

Detergent activated virions of the type C retrovirus SSV-1/SSAV complex were used to synthesize single stranded cDNA. This material was made double stranded using DNA polymerase I, the ends were flattened using S1 nuclease, and poly dC tails were added with terminal deoxynucleotide transferase. The product was inserted into pBR322 by annealing to poly dG tails added at the plasmid's Pst I restriction site and propagated in E. coli X1776. A 905 ±10 base pair fragment including the simian virus "strong stop" sequence, plus adjacent sequences, was obtained. Mapping and sequence data will be presented. Labeled probes made from this plasmid are presently being used to screen human tumor cell DNA's for regions of homology.

617 STRUCTURE AND BIOLOGICAL ACTIVITY OF AVIAN SARCOMA VIRUS DNA CLONED IN BACTERIOPHAGE LAMBDA, Peter Highfield, Lori Rafield, Tona Gilmer and J. Thomas Parsons, University of Virginia, Charlottesville, Va. 22908

Unintegrated Prague A ASV proviral DNA has been cloned in bacteriophage lambda Covalently-closed circular DNA, purified from Prague A-infected quail cells, was subjected to partial Eco RI cleavage, ligated to the Eco RI "arms" of λgtWES λB, packaged into virions and recombinant progeny identified by hybridization with labeled viral complementary DNA. Among the recombinants was one with the following structure



This recombinant is virtually co-linear with the genomic RNA, differing only in that the 3'-terminal 60 nucleotides present in the genomic RNA are now present at the 5' end of the cloned viral DNA sequence. The structure and biological properties of this recombinant will be discussed.

618 CHARACTERIZATION OF THE "src" GENE OF MOLONEY MSV 124, Dino Dina, Ed Benz, Steve Baum, Russell Medford, Lenny Garfinkel and Bernardo Nadal-Ginard, Albert Einstein College of Medicine, Bronx, New York 10461

Moloney MSV virions have been shown to synthesize several discrete forms of DNA in vitro. These include full genome length ds DNA, circular DNA, a deleted linear DNA missing the 5' end 600 bp repeat and a 600 bp repeat unit. All these forms have been cloned in pBR 322. Moreover, subclones containing the "src" region of the genome have been obtained. Sequencing of both the repeat unit and the sarcoma specific region is in progress.

These cloned vDNA molecules have been used to analyze the formation of viral mRNA in vivo. We have shown that spliced mRNA-like molecules are present in virions and cellular cytoplasms. The src mRNA, about 20S in size has been purified by hybridization to cloned DNA and characterized by electron microscopy and S_1 mapping. Preliminary data indicate that the purified cellular vRNAs can be translated into sarcoma-specific polypeptides which are also made by virion RNA.

619 SARC: A NORMAL MOUSE CELL SEQUENCE HOMOLOGOUS TO THE "SRC" SEQUENCE OF MOLONEY SARCOMA VIRUS. Marianne Oskarsson, William L. McClements, Donald G. Blair, Jakob V. Maizel and George F. Vande Woude. National Institutes of Health, Bethesda MD 20205

Moloney sarcoma virus (MSV) contains a sequence (src) presumably acquired from the host during passage of the parent Moloney murine leukemia virus through Balb/c mice. This sequence is apparently a prerequisite for the tumorigenic potential of the virus and its ability to morphologically transform cells in vitro. Using recombinant DNA techniques we have cloned in phage lambda a sequence (sarc) from Balb/c mouse DNA homologous to the MSV src sequence. The cloned DNA fragment was compared to two cloned integrated proviruses of MSV (the ml and HT1 isolates) by heteroduplex mapping and restriction endonuclease analyses. Only one region of homology was observed corresponding to the src region of the proviruses and measuring 1.17 kb for ml and 1.3 kb for HT1 MSV. Nine identical restriction sites were mapped in Balb/c sarc and HT1 MSV src. The cloned fragment also contains 14 kb of mouse cell sequences flanking sarc, which showed no homology to other regions of MSV. The sarc fragment was tested in a direct DNA transfection assay for its ability to induce foci of transformation on NIH-3T3 cells. In this assay the cloned provirus of HT1 MSV produces 4.7×10^4 focus forming units per pmole DNA. Subgenomic fragments of MSV also transform NIH-3T3 cells. However, in repeated transfections even at high DNA concentrations the sarc fragment or subcloned fragments containing sarc were inactive in the assay.

620 MOLECULAR CLONING AND CHARACTERIZATION OF ENDOGENOUS SARC GENES FROM MOUSE FIBROBLASTS, Matt Jones, Robert Bosselman, Frans van der Hoorn, Ton Berns and Inder M. Verma, Tumor Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, California 92138

We have identified a 14.0 kb and a 5.5 kb Eco R_I fragment from uninfected mouse fibroblasts which hybridize to molecularly cloned DNA representing the src-specific region of Moloney mouse sarcoma virus (M-MSV). Both the 14.0 kb and the 5.5 kb fragments appear to contain the entire M-MSV src-specific region. The 14.0 kb Eco R_I fragment was cloned in lambdoid phage, Charon 4A, and characterized by restriction endonuclease mapping. When both the cloned unintegrated M-MSV DNA and 14.0 kb endogenous sarc gene are cleaved with restriction endonuclease Hind III and Bgl I, a fragment of 1000-1100 nucleotides is generated, which hybridizes to M-MSV src-specific DNA. Heteroduplexes formed between cloned endogenous sarc gene and M-MSV genomic RNA share a region of homology of about 1.0 kb. Hybridization of cloned endogenous sarc DNA to M-MSV genomic RNA, followed by digestion with S1 nuclease also shows a DNA fragment of about 1.0 kb long. The 5.5 kb Eco R_I fragment is being cloned. The origin of M-MSV src from endogenous sarc gene will be discussed.

621 BIOLOGICAL ANALYSES OF THE TRANSFORMING ACTIVITY OF CLONED RECOMBINANT MOLONEY MSV, D.G. Blair, W.L. McClements, M.A. Oskarsson, G. Vande Woude, and P.J. Fischinger, Laboratories of Viral Carcinogenesis and Molecular Virology, NCI, NIH, Bethesda, MD 20205.
Moloney sarcoma virus (MSV) arose in Balb/c mice as a recombinant, with cellular sequences (src) linked to viral sequences derived from the parent leukemia virus (leuk). We have cloned the integrated provirus of two MSV isolates, together with flanking cellular sequences, from transformed mink cells into bacteriophage λ (1), and have subsequently cloned subgenomic MSV fragments into the plasmid pBR322. Utilizing these cloned DNA's in transfection assays, we have attempted to define the role of specific viral sequences in transformation.

Cloned whole proviral DNA transfects and transforms with high efficiencies (~5×10^4-8×10^4 ffu/pmole), and in the majority of cases these cells contain rescuable MSV. Cloned src-containing subgenomic fragments, from which either the terminal 5' or 3' leukemia and mink-derived sequences were deleted, also transform with only a 6-fold reduced efficiency. Similar clones which lack both terminal leuk sequences (pHTL10) transform with efficiencies ~6000-fold lower than cloned whole MSV genome. Activity can be restored by the in vitro addition of non-transforming proviral sequences which contain the 600 bp terminal redundancy (TRS). Furthermore, co-transfection with the poorly transforming pHT10 fragment together with a non-transforming clone containing only TRS and associated mink cell sequences transforms 50-100 fold more efficiently than pHT10 alone. Collectively, the data indicate that sequences in addition to src are necessary for efficient cell transformation and that TRS may function to enhance the insertion and/or expression of transforming sarcoma viral sequences.
(1) Vande Woude et al., PNAS 76: 4464-4468 (1979).

622 TRANSFORMATION BY MOLECULARLY CLONED HARVEY MURINE SARCOMA VIRUS DNA, Esther H. Chang and Douglas R. Lowy, Dermatology Branch, NCI, NIH, Bethesda, MD 20205.
Supercoiled Harvey murine sarcoma virus (Ha-MuSV) DNA, linearized at its unique Eco RI site, has been molecularly cloned in λ-phage. The cloned Ha-MuSV DNA is therefore permuted with respect to its structure in virus-transformed mammalian cells. Both intact and Eco RI digested λ.Ha-MuSV DNA induced foci of transformed mouse cells with similar efficiency. Transformed cell lines were established from independently induced foci. Superinfection with a helper-independent virus rescued Ha-MuSV with low efficiency ($<10^3$ ffu/ml) from all lines transformed by intact λ.Ha-MuSV DNA and from most lines transformed by Eco RI digested DNA. Virus was rescued with high efficiency ($>10^6$ ffu/ml) from the remaining lines transformed by digested DNA and from all lines non-productively transformed by Ha-MuSV virus. Southern blotting of high molecular weight DNA from low-rescue lines indicated that they contained Ha-MuSV only in the permuted orientation, while high-rescue lines transformed by Eco RI digested DNA contained a head-to-tail dimer of the cloned viral DNA. All transformed lines contained high levels of both the Ha-MuSV p21 and of RNA sequences derived from the 5' side of the viral Eco RI site. Only the high-rescue lines contained high levels of RNA sequences from the 3' side of the viral Eco RI site; the low-rescue lines contained little or no RNA of this class. These results suggested that, in contrast to Rous sarcoma virus and Moloney-MuSV, the transforming region (and the sequences coding for the p21) of Ha-MuSV is contained within the 5' half of the viral genome. Infectivity studies utilizing defined subgenomic fragments of Ha-MuSV DNA have directly confirmed this hypothesis.

623 CHARACTERIZATION OF MOLECULARLY CLONED SPLEEN FOCUS-FORMING VIRUS DNA, David L. Linemeyer, Sandra K. Ruscetti, John G. Menke, and Edward M. Scolnick, National Cancer Institute, Bethesda, MD 20205
Unintegrated supercoiled spleen focus-forming virus (SFFV) DNA was extracted from rat cells newly infected with a woolly monkey virus pseudotype of the Lilly-Steeves strain of SFFV. The DNA was linearized by digestion at the unique Hind III endonuclease site, inserted into the plasmid pBR322, and cloned in an approved EK2 host. Six independent clones containing SFFV DNA inserts were isolated and analyzed by restriction endonuclease digestion. One clone had an insert of 5.7 kbp with the same restriction enzyme sites as the unintegrated linear SFFV DNA except that it lacked a copy of the terminally redundant sequence. Recombinant DNA isolated from this clone was transfected into NIH 3T3 cells and rescued either by co-transfection of cloned Friend murine leukemia helper virus (F-MuLV) DNA or by superinfection with a non-erthroleukemia-inducing variant of F-MuLV. Inoculation of adult NIH Swiss mice with the virus produced from these transfections induced an erythroleukemia, characterized by splenomegaly, polycythemia and spleen foci, which was identical to that induced by the original F-MuLV pseudotype of SFFV. Also, the SFFV-specific gp52 protein was synthesized both in the transfected fibroblasts as well as the bone marrow of the inoculated mice. The recombinant DNA was infectious whether or not it was digested with Hind III endonuclease but was not infectious after digestion with Eco RI endonuclease suggesting possible sites for the transforming gene.

624 TRANSFORMING GENE OF MURINE SARCOMA VIRUS, Steven R. Tronick, Keith C. Robbins, Eli Canaani, Sushilkumar G. Devare, Philip R. Andersen, and Stuart A. Aaronson, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda MD 20205

The Moloney isolate of murine sarcoma virus (MSV) is representative of replication-defective mammalian transforming RNA viruses. In order to define in detail the structure and origin of the regions of the MSV genome specific for transformation, circular 5.8 kbp MSV (strain 124) DNA was isolated from infected cells and molecularly cloned using a bacteriophage λ vector. Five different sized MSV-specific inserts were found (6.4, 5.8, 5.2, 4.0, and 2.0 kbp, respectively). Several plaques contained more than one size class insert; and following propagation of certain phage stocks, the smaller sized inserts accumulated. Heteroduplex and restriction enzyme analyses of the various sized inserts indicated that the 6.4 kbp molecule contained in tandem three of the 0.6 kbp redundant sequences known to be at the termini of the linear MSV DNA, and the 5.8 and 5.2 kbp species arose by sequential deletion of these sequences. The presence of the two smaller forms indicates the presence of additional direct repeats in the MSV genome. Xba I and Hind III digestion of MSV DNA generates a fragment specific for the non-helper virus related sequences of MSV. This fragment was isolated from cloned MSV DNA, labelled, and hybridized to size fractionated normal mouse DNA treated with either EcoRI (which does not cut MSV DNA) or Xba I plus Hind III in order to examine the degree of representation and arrangement of sarcoma virus related sequences within the cell. The implications of the results regarding the structure and origin of the transforming gene of MSV will be discussed.

625 CLONING AND ISOLATION OF RETROVIRUS SPECIFIC SEQUENCES FROM NORMAL AND TRANSFORMED MOUSE CELLS, Eric James and Robert T. Garvin, University of Kentucky Lexington, Kentucky 40356

A DNA library has been prepared from Cl 124 mouse cells. DNA isolated from MUS cells was cleaved with Eco RI and ligated into DNA cloning arms prepared from the bacteriophage vector Charon 4A. Chimeric phage were packaged in vitro and amplified in DP50 Sup F. The library and one prepared from a random bred mouse has been screened by in situ hybridization of M MuSV cDNA to nitrocellulose filters carrying DNA transferred from phage plaques on mega plates (33 x 42 cm). Four retrovirus specific clones have been identified. DNA carried in these clones has been digested with Eco RI and resolved by electrophoresis through 0.6% agarose gel and the DNA transferred to nitrocellulose filters by blotting using the Southern technique. The DNA inserts hybridized specifically to M Mu SV cDNA probe. MUS DNA insert DNA has been prepared by preparative electrophoresis in SeaPlaque agarose gel and subjected to restriction analysis. Nucleic acid sequence analysis of specific regions of the MUS inserts is underway using DNA fragments cloned in M13p5 and the dideoxy protocol.

626 THE GENETICS OF AVIAN RNA TUMOR VIRUSES INDUCING OSTEOPETROSIS. Emmett V. Schmidt, Jack D. Keene, Maxine Linial, and Ralph E. Smith. Department of Microbiology, Duke University Medical Center; Durham, N.C.; and University of Washington; Seattle, Washington.

Avian leukosis viruses have been shown by hybridization studies to differ in 15-20% of sequences concentrated at the 3' end of the genomic RNA molecule. In order to characterise the nature of this variation, we have concentrated on viruses that induce osteopetrosis, a proliferative disorder of bone. These viruses include RAV-1(O), RAV-2(O), RAV-2(N), ARC, and RAV-50. Additional viruses were generated in this study by recombination: PrBtd1? was the product of a cross between RAV-2(O) and PrE which was subsequently made defective for transformation; and BF-10 was the product of a cross between RAV-2(O) and RIV, the nononcogenic endogenous pheasant virus. Both viruses induced osteopetrosis.

The genomes of these viruses were analysed for their T1 oligonucleotide fingerprints. The recombinants and their parents were studied for the location of the crossover. The other viruses were used to confirm the nature of subgroup differences and regions of homology general to leukosis viruses. RAV-0 was examined for the oligonucleotides which were not important to oncogenicity. The oligonucleotides of RAV-2(O) were ordered within the genome and the order was found to agree with previously published maps of the RNA tumor viruses. An oligonucleotide located near the 3' end of the map seemed to correlate with induction of osteopetrosis.

627 THE GENOMIC RNA OF DEFECTIVE AVIAN RETICULOENDOTHELIOSIS VIRUS, Robert S. Cohen, Martin L. Breitman and Michael M.C. Lai. Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033

Avian reticuloendotheliosis virus (REV) is a defective acute leukemia virus which can transform both avian bone marrow cells and fibroblasts. It requires a helper virus (REV-A) for its replication. The genome of REV is a 28S (2 x 10^6 daltons) RNA while its helper virus has a 34S (3 x 10^6 daltons) RNA. The small size of the REV genome suggests deletion as the reason for its defectiveness. However, complementary DNA made from helper REV-A protects only 70% of the 28S REV RNA suggesting the presence of REV-specific "transforming" gene sequences. Fingerprinting analysis of REV RNA showed that more than half of the large RNase T_1-resistant oligonucleotides from REV RNA are specific for REV. These oligonucleotide sequences are not shared with sarcoma viruses or other acute leukemia viruses, suggesting that the specific sequences of REV may represent a new class of oncogenic genes. These specific sequences are mapped in the middle of the 28S RNA and may code for at least one REV-specific transforming protein, p58.

628 GENETIC STRUCTURE AND TRANSFORMING GENES OF AVIAN ACUTE LEUKEMIA VIRUSES
Klaus Bister and Peter H. Duesberg, Dept. of Molecular Biology, University of California, Berkeley, California 94720

RNA structure and protein products of avian acute leukemia viruses have been studied, including three members of the MC29 subgroup of avian oncogenic viruses (MC29, CMII, MH2) and avian erythroblastosis virus (AEV). All of these viruses are defective for replication and have broad oncogenic spectra for transformation in vivo (sarcomas, carcinomas, erythroid and myeloid leukemias) and in vitro (transformation of cultured avian and mammalian fibroblasts and proliferation and alteration of hematopoietic cells in culture). They all share a basic genetic structure of their RNA genome. It measures between 5.5 and 6.0 kilobases (kb) and three RNA segments can be distinguished by oligonucleotide mapping: (1) a 5' gag-related sequence of about 1-1.5 kb; (2) an internal MC29 subgroup- or AEV-specific segment of 2-3 kb, which is unrelated to nondefective viral RNAs or to src; (3) a 3' group-specific (env-related) segment of 1.5-2.5 kb. Comparative analysis of the acute leukemia viral RNAs suggests that the genetic units consisting of the 5' gag-related and the internal MC29 subgroup- or AEV-specific RNA segments serve a primary function in oncogenicity. They appear to represent the onc genes of these viruses and code for gag-related, nonstructural and probably transforming polyproteins of MW 75-110,000 daltons. Specific src-unrelated RNA sequences in the MC29 subgroup and in AEV, which correlate with oncogenicity, suggest that multiple onc genes exist in the avian tumor virus family.

629 PROVIRAL GENOMES OF THE AVIAN MYELOBLASTOSIS VIRUS COMPLEX. D. G. Bergmann and M. A. Baluda, UCLA School of Medicine and Molecular Biology Institute, Los Angeles, California 90024.

The avian myeloblastosis virus complex (AMV-S) consists of at least 3 viruses: a presumably defective leukemogenic agent and two myeloblastosis associated helper viruses; MAV-1 and MAV-2. The size of the unintegrated linear double-stranded viral DNA genomes has been determined and Eco Rl and Hind III sites have been mapped in the viral DNA. The size of the DNA genome is the same for both MAVs (5.3 X 10^6 daltons), but the two MAVs differ in their Hind III recognition sites. Also, the MAV genomes contain terminal redundancies of approximately 300 base pairs. Studies of the structure and location of the integrated proviral genomes in cloned MAV infected CEF show that the MAV proviral DNA integrates colinearly with respect to the unintegrated viral DNA and at many sites within the chicken genome. All clones produced virus regardless of the sites of integration.

Both MAV-1 and MAV-2 genomes are present in AMV-S but MAV-2 appears to be a minor component. Our data also indicate the existence of another DNA species of slightly smaller size (4.8 X 10^6 daltons) than the MAVs. This smaller genome also comprises a minor portion of the AMV-S complex and generates different DNA fragments after Eco Rl or Hind III treatment. This smaller DNA species is specifically associated with leukemic myeloblasts, and is presumably the leukemogenic genome of AMV-S (Souza et al., in press).

630 THE PRESUMPTIVE LEUKEMOGENIC GENOME OF THE AVIAN MYELOBLASTOSIS VIRUS COMPLEX. L. M. Souza and M. A. Baluda. UCLA School of Medicine and Molecular Biology Institute, Los Angeles, California 90024.

One of the neoplasias induced in chickens by standard avian myeloblastosis virus (AMV-S) is acute myeloblastic leukemia. Southern blot analysis of Eco Rl and Hind III digested leukemic myeloblast DNA show: 1) Specific proviral fragments are present in all leukemic myeloblasts regardless of the chicken genetic background, 2) These AMV specific fragments are not found in cells infected by helper virus alone, and 3) The same AMV specific fragments are present regardless of the AMV pseudotype used. Analysis of DNA from colonies arising from single leukemic myeloblasts (containing or not containing the helper genome) permitted the identification of restriction endonuclease fragments which belong to either the helper or the presumptive AMV genome. Furthermore, the juncture fragments of the clonal DNAs show that both AMV and its helper(s) can integrate at different cellular DNA sites. Two λ Charon 4A clones containing proviral DNA from leukemic myeloblast have been characterized by restriction endonuclease digestion. Clone λ 10A2-1 contains 85% of the helper genome as well as host sequences flanking the 5' end of the genome with respect to the viral RNA. Clone λ 11A1-1 contains an entire presumptive AMV genome with flanking chicken sequences. The size of the putative AMV genome is approximately 4.8 md compared to approximately 5.25 md for its natural helper(s). The difference in size between AMV and its helper(s) is relatively small compared to other defective leukosis viruses relative to their helper(s), i.e., MC29 is approximately 3.4 md while its helper is approximately 5.2 md. The nature of the defect(s) in the AMV genome rendering it both apparently defective and leukemogenic is under investigation.

631 IDENTIFICATION AND COMPARISON OF DEFECTIVE AND NONDEFECTIVE COMPONENTS OF THE ANEMIA AND POLYCYTHEMIA-INDUCING STRAINS OF FRIEND VIRUS. Leonard Evans, Michael Nunn, and Peter Duesberg, University of California, Berkeley, CA 94720; David Linemeyer, David Troxler, and Edward Scolnick, National Cancer Institute, Bethesda, MD 20014

The defective RNA components of the anemia-inducing (FV-A) and the polycythemia-inducing strains of the Friend erythroleukemia virus have been identified and compared to the RNAs of the helper Friend murine leukemia virus (Fr-MLV) and a dualtropic mink cell focus-inducing variant of Fr-MLV (MCF Fr-MLV). The replication-defective genomes of FV-P and FV-A, termed here FVP and FVA (unhyphenated), correspond to RNAs of approximately 7.4 and 6.6 kilobases (kb), respectively, compared to the approximately 9.6 kb RNAs of Fr-MLV and MCF Fr-MLV. All RNase T_1-resistant oligonucleotides of FVP and most T_1-oligonucleotides of FVA have identical or closely-related sequences in MCF Fr-MLV. These include Fr-MLV-related sequences as well as sequences specifically related to MCF Fr-MLV. T_1-oligonucleotide mapping experiments revealed that the basic structure of FVP RNA, as well as FVA RNA, is that of a deletion mutant of MCF-Fr-MLV; however, numerous smaller differences distinguish the related sequences of the two RNAs. These results suggest that the onc gene of FVP (and probably of FVA) is comprised of sequences closely related to a replication competent MLV. This is in contrast to the onc genes of other defective, rapidly transforming RNA tumor viruses which include a major specific sequence unrelated to the genes of helper viruses. Since MCF Fr-MLV does not induce the rapid onset of erythroleukemia observed with FVP or FVA, the oncogenic activity of these replication-defective viruses must be the result of the genetic alterations described above.

632 RADIATION LEUKEMIA VIRAL RNAs, Jungsuh P. Kim, Simone Manteuil-Brutlag and Henry S. Kaplan, Cancer Biology Research Laboratory, Department of Radiology, Stanford University School of Medicine, Stanford, CA 94305

We have analyzed the RNA genome of RadLV/VL_3, a highly oncogenic murine leukemia virus which is produced by a permanent cell line derived from a RadLV-induced thymic lymphoma of C57BL/Ka mice. Two distinct RNA components were found in the virions: a 70S dimer containing two 8 kb RNA subunits and a 54S dimer containing two 5.6 kb RNAs. A non-oncogenic retrovirus, BL/Ka(B), endogenous in the same strain of mice, contains only 8 kb viral RNA subunits. We made long strands of RadLV/VL_3 cDNA in the absence of actinomycin D. Two discrete DNA molecules, 9.0 kb and 5.6 kb in length were synthesized. By hybridizing these DNA products with an excess of purified 5.6 kb RadLV/VL_3 RNA, followed by treatment with S1, we showed the two RNA subunits share 2.3 kb of their sequences.

633 EVIDENCE OF DISEASE-SPECIFIC SEQUENCES PRESENT ONLY IN GENOMES OF ONCOGENIC GaLV. Lily Sun and Thomas G. Kawakami, Comparative Oncology Laboratory, University of California, Davis, California, 95616.

A type-C virus isolated from a gibbon with myelogenous leukemia, GaLV-3M, is oncogenic for host animals since the same disease has been induced in several gibbons. A similar virus isolated from a clinically normal gibbon, GaLV-5, is not oncogenic for host animals since gibbons infected by this virus remained persistently viremic for several years without leukemia. To define genetic factors that could be responsible for the biological differences between GaLV-3M and GaLV-5, we analyzed the genomes of these viruses by molecular hybridization. The genome of GaLV-3M was separated into four subgenomic fractions by a sequential adsorption procedure using cellular DNA carrying proviral sequences of GaLV associated with various forms of leukemia. One fraction was found to contain RNA sequences identifiable only with myelogenous leukemia and designated as "MYE". In an attempt to determine if these disease-specific "MYE" sequences are present in genome of GaLV-5, the genome of GaLV-3M was adsorbed by cellular DNA carrying proviral sequences of GaLV-5 and separated into sequences homologous to and sequences non-homologous to GaLV-5. The RNA of the non-homologous fraction can readily detect high levels (46-52%) of complementary DNA sequences only in myelogenous leukemic tissues. This indicates that the major portion of the "MYE" RNA is contained in the non-homologous fraction of GaLV-3M and thus not in genome of GaLV-5. The lack of the disease-specific sequences in genome of GaLV-5 may account for its apparent non-oncogenicity so that animals infected by GaLV-5 remained persistently viremic without development of leukemia.

634 **PARTIAL CHARACTERIZATION OF A 23,000 DALTON (P23) POLYPEPTIDE ENCODED BY MOLONEY MURINE SARCOMA VIRUS (Mo-MuSV), R. B. Arlinghaus, D. D. Lyons, E. C. Murphy, Jr., and S-M. Mong. University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030**

RNA from Moloney murine sarcoma virus (Mo-MuSV) produced in clone G8-124 infected cells was isolated and translated in a mRNA-dependent cell-free protein synthesis system. The cell-free product of both 50-70S genome-sized RNA and 30S subunit RNA contained four major polypeptides with apparent molecular weights of 63,000 ($P63^{gag}$), 42,000 (P42), 38,000 (P38), and 23,000 (P23). $P63^{gag}$ was translated primarily from full-size subunit RNA, while P38 and P23 were preferentially translated from 18-25S poly(A)-containing subgenomic RNA. $P63^{gag}$ contained antigenic determinants and tryptic peptides characteristic of Moloney murine leukemia virus (Mo-MuLV) p15, pp12, and p30. Although $P63^{gag}$ was recognized by anti-p10, its tryptic map showed that it lacked a p10-characteristic tyrosine-containing tryptic peptide. P42 and P38 also contained antigenic determinants and tryptic peptides of p15, pp12, and a portion of p30, as well as tryptic peptides that appeared to comigrate with some of the P23 tryptic peptides. Analyses of P23 clearly demonstrated that it lacked antigenic determinants and tyrosine-containing tryptic peptides of the viral core proteins, reverse transcriptase, and the 'env' gene product. Hence, the results suggest that P23 may possibly be the product of the Mo-MuSV acquired sequences. Studies with a temperature sensitive transformation mutant derived from a subclone of Mo-MuSV-124 have identified a P85 that contains p15, pp12 and part of p30 plus additional sequences. The relationship of these additional sequences to P23 is under investigation.

635 THREE INDEPENDENT ISOLATES OF FELINE SARCOMA VIRUS CODE FOR THREE DISTINCT GAG-X POLYPROTEINS, Sandra Ruscetti, Lubomir Turek and Charles Sherr, National Cancer Institute, Bethesda, MD 20205.

Three isolates of feline sarcoma virus (FeSV) are each recombinants between feline leukemia virus (FeLV)-specific sequences and other sarcoma-specific sequences (src) transduced from normal cat cells. Each strain of FeSV codes for a "gag-X" polyprotein which contains antigens related to amino-terminal gag gene products as well as unique antigenic determinants (designated X). Studies with molecularly cloned Snyder-Theilen (ST) FeSV have shown that the X moiety is encoded by src sequences. To determine the differences between the "gag-X" polyproteins of the Gardner-Arnstein (GA), McDonough (M) and ST strains, we raised antisera to FeSV-transformed nonproducer cells in rats and absorbed the sera with FeLV to remove antibodies to gag gene products. Selected absorbed sera contained high titer antibodies to X. Using metabolic labeling and immune precipitation, each strain of FeSV was found to code for a unique product. The molecular weights of these polyproteins are 78,000, 85,000 and 130,000 for the ST, GA and M strains, respectively, and all are phosphorylated. Using antisera to the X moiety of each polyprotein, all were found to contain unique antigenic determinants characteristic of the FeSV strain. Thus, antisera directed against the putative src gene products of the three FeSV isolates can be used to confirm their independent origin and to characterize the nature of proteins presumably involved in cell transformation.

636 FELINE SARCOMA VIRUS-CODED POLYPROTEINS, Mariano Barbacid and Sushilkumar G. Devare, National Cancer Institute, Bethesda, Maryland 20205

The McDonough (SM), Gardner-Arnstein (GA) and Snyder-Theilen (ST) strains of feline sarcoma virus (FeSV) code for high molecular polyproteins that contain varying amounts of the amino terminal region of the FeLV gag gene-coded precursor protein and a polypeptide(s) of an, as yet, undetermined nature. Antisera specific for the FeLV and FeSV-specific domains of each of these polyproteins have been obtained from goats immunized with purified polyproteins or autologous FeSV-transformed cells as well as from tumor bearing animals. These antisera, along with tryptic peptide fingerprinting analysis techniques, have been utilized to characterize these FeSV-coded polyproteins. The SM-FeSV primary translational product is a 180,000 dalton polyprotein which is processed into an unstable 60,000 dalton molecule containing the p15-p12-p30 fragment of the FeLV gag gene-coded precursor protein and a 120,000 dalton FeSV-specific polypeptide. The GA- and ST-FeSV genomes code for relatively stable polyproteins (half-lives around 16 hours) of 95,000 and 85,000 daltons, respectively, which in addition to the amino terminal moiety, also contain FeSV specific polypeptides. The latter appeared to be antigenically cross-reactive and exhibited common methionine-containing peptides. Finally, evidence indicating a cellular origin for the sarcoma-specific moiety of the FeSV-coded polyproteins will be presented.

637 VIRUS - SPECIFIC PRECURSOR POLYPEPTIDES IN SIMIAN SARCOMA VIRUS (SSV) - TRANSFORMED PRODUCER AND NONPRODUCER PRIMATE CELLS, Carolyn M. Bergholz, Department of Microbiology, University of Illinois, Urbana, Illinois 61801.

Studies of the synthesis and processing of viral proteins in simian sarcoma associated virus (SSAV) - infected and SSV(SSAV) - transformed marmoset and human cell lines has demonstrated polyprotein precursors precipitable by anti-SSAV p30 and anti-SSAV gp70. A 60,000 dalton precursor polypeptide (pr60) and lesser amounts of 52,000 and 37,000 dalton polypeptides are precipitable from ^{35}S-methionine-labeled cell extracts by anti-p30 serum Immunoprecipation by anti-gp70 revealed large amounts of intracellular gp70 precursor of 80,000 daltons. Precursor - product relationships and post-translational modification of gag and env precursors will be discussed. Clonal transformed cell lines (HF/SSV-NP) infected with SSV in the absence of SSAV were evaluated for expression of virus-specific proteins precipitable by antisera prepared against SSAV p30, gp70 and tween-ether disrupted virus. No p30 and no polypeptides precipitable by anti-SSAV gp70 were detected in any of the cell lines. Polyprotein precursors precipitable by anti-SSAV p30 were identified in some HF/SSV-NP clonal cell lines but absent in others. Results of studies comparing proteins precipitated from extracts of normal HF, untransformed HF/SSAV and HF/SSV-NP transformed cell lines by antisera from marmosets bearing SSV-induced tumors will be discussed.

638 LEUKEMIA-SPECIFIC SEQUENCES OF AVIAN ERYTHROBLASTOSIS VIRUS, Michael M.C. Lai, James C. Neil, Timothy Wong and Peter K. Vogt, Department of Microbiology, University of Southern California School of Medicine, Los Angeles, CA 90033

Avian erythroblastosis virus (AEV) is an avian acute leukemia virus which can transform both bone marrow cells and fibroblasts in tissue culture. It has a 28S RNA genome. Previous data obtained from DNA·RNA hybridization, oligonucleotide fingerprinting and heteroduplex mapping showed that more than half of the 28S RNA represented AEV-specific transforming gene sequences which occupied a 3.25 kb contiguous segment in the middle of the 6 kb AEV genome. *In vitro* translation of AEV RNA showed that these AEV-specific transformation sequences code for two proteins p75 and p40. The former is a gag-containing polyprotein, and the latter contains unique peptides not shared with other viral proteins. Which of these two proteins is directly responsible for leukemogenesis is not clear.

The AEV-specific sequences were present in the DNA of normal chicken cells as well as a variety of mammalian cells. The chromosomal location of these sequences in the chicken cells will be discussed.

639 IN VITRO TRANSLATION OF MSV VIRION RNA, Jackie Papkoff, Tony Hunter and Inder Verma, The Salk Institute, Post Office Box 85800, San Diego, California 92138.

Moloney murine sarcoma virus (Mo-MSV) presumably arose by recombination between Mo-MLV and mouse genetic material. These acquired sequences are contained within the sarcomagenic portions of the Mo-MSV genome, which is defined as the viral src gene. To identify the gene products of this region, Mo-MSV-clone 124 virion RNA was translated in the messenger-dependent reticulocyte lysate and the products were characterized by immunoprecipitation, tryptic peptide mapping and hybrid-arrest translation. The major in vitro translation products have apparent molecular weights of 62K, 37K, 33K, 24K, and 18K. The 62K protein, on the basis of both immunoprecipitation and hybrid-arrest translation with cloned recombinant DNA containing gag gene but no src gene sequences, is encoded by the gag gene at the 5' end of the Mo-MSV genome. The 37K, 33K, 24K and 18K proteins which form an overlapping set by tryptic peptide mapping are not recognized by antiserum against gag or env proteins and can be synthesized from polyadenylated fragments containing only the 3' src region of Mo-MSV genomic RNA. In addition, the synthesis of this family of proteins, but not of the 62K protein, can be inhibited by prehybridizing virion RNA with cloned recombinant DNA containing only Mo-MSV src sequences. We have extended these studies to Gazdar MSV, an independent isolate containing a similar src gene. In vitro translation of Gazdar MSV virion RNA results in the synthesis of a group of related proteins similar in both size and peptide composition to the 37K family seen with Mo-MSV. Experiments are underway to identify the RNA(s) and protein(s) derived from the MSV src gene in transformed cells.

640 FRIEND VIRUS: SFFV SPECIFIC GENE EXPRESSION, D. Frisby, J. Bilello*,G. Colletta, G. Warnecke*,G. Koch*,I. Pragnell and W. Ostertag, Beatson Inst., Glasgow G61 1BD, Scotland. *Dept. Mol. Biol. ,Univ. Hamburg, D-2000 Hamburg 13, West Germany. Cloned virus from a Friend erythroleukaemia cell line producing an excess of the defective spleen focus-forming virus (SFFV) was used to isolate SFFV non-producer cell-lines. These cell lines were analysed for viral gene expression by both immuno-precipitation and molecular hybridisation techniques. The major molecular species precipitated with anti-envelope glycopretein (gp70) sera in both erythroid and non-erythroid cell lines was a glycoprotein of 55,000 molecular weight (gp55). This protein (gp55) is present in Friend cells transformed by either the anaemia (Fv-A) or the polycythaemia (Fv-P) inducing Friend virus but is not detectable in cells infected with the helper virus alone. A Rauscher virus transformed erythroleukaemia cell line (RA-1) and the Friend cell line, T-3C12 express a related but 52,000 molecular weight protein. A chemically induced rat and erythroleukaemia cell line (D5A1) was negative for both env related gene products and SFFV related RNA. The non-producer SC-1 cell lines were negative also for gag gene specified proteins. An absolute correlation was established between SFFV-specific RNA and gp55 expression. SFFV+ LLV- cells express both 32S and 21S RNA species related to Friend virus. SFFV+ LLV- cells expressing 21S SFFV-specific RNA constitutively synthesise gp55. These data indicate that gp55 is coded for by the SFFV genome and suggest furthermore that it is encoded by a sub-genomic SFFV mRNA species.

641 CHARACTERIZATION OF FRIEND AND RAUSCHER NONPRODUCER CELLS AND IMMUNOSELECTION OF MUTANTS, Martin Ruta and David Kabat, Univ. Ore. Health SCI Ctr, Portland, OR 97201

The Friend and Rauscher murine erythroleukemia viral complexes contain at least two biologically active viral components. We have cloned into fibroblasts replication defective viruses (SFFV) which cause a rapid erythroleukemia when injected with a helper virus in susceptible mice. The SFFV encoded proteins expressed in the nonproducer cell lines by the different Friend and Rauscher SFFV clones were compared. We determined that SFFV nonproducer clones exhibit heterogeneity in gag gene expression. However, each viral isolate encodes a glycoprotein of approximately Mr 55,000 (gp55) which is immunologically related to the gp70s of dual tropic MuLVs. The encoding of a similar protein by both the Friend and Rauscher SFFVs is consistent with the possibility that gp55 might be the SFFV transforming protein. In addition, the nonproducer cell lines express some gp55 on their surfaces. We are trying a new approach to isolate viral mutants. Nonproducer SFFV infected cells are immunoselected using cytotoxic anti-gp70 serum and complement. Many resistant cell lines have been isolated which contain abnormal intracellular forms of gp55 and which lack cell surface gp55. We are examining these resistant cell lines in order to determine if the defects in gp55 processing are caused by cellular or by viral mutations. Viral mutants will then be screened for leukemogenicity.

642 Biological and Biochemical Characterization of Transformation Mutants of Rous Sarcoma Virus Which Induce a "Partially" Transformed Phenotype. Michael J. Weber and Deborah D. Anderson, Department of Microbiology, University of Illinois, Urbana, IL 61801. We will present evidence that the complex array of cellular alterations which constitute the "transformed phenotype" result from interaction of the transforming src gene product with more than one primary target. Our reasoning is as follows: if $pp60^{src}$ has more than one primary cellular target, it should be possible to isolate mutants of RSV which affect some targets but not others. Such mutants should have the phenotype of "partial dissociation" mutants. Three such mutants have been isolated and characterized. CU2 induces a novel "blebby" morphology, and only tiny soft agar colonies. tsCU11 has little if any effect on cellular morphology, but causes soft agar colonies as large as wild-type virus at 36°C. CU12 induces a "fusiform" morphology and giant soft agar colonies. The mutant-infected cells have also been characterized with respect to hexose transport, adhesiveness, fibronectin, saturation density, focus formation and plasminogen activator. The pattern of dissociations of these parameters is most consistent with a model in which $pp60^{src}$ interacts with two or more primary targets.

The partial characterization of the $pp60^{src}$ from these mutants has been started. All the mutants code for $pp60^{src}$ which is similar to wild-type in amount, molecular weight and phosphorylation. However, in addition, CU12-infected cells possess a 62,000 Dalton phosphoprotein which is precipitated by anti-tumor antiserum but not by antisera raised against whole virus or purified virus structural proteins.

643 A SINGLE GENE MUTATION IN ROUS SARCOMA VIRUS INDUCES A HEAT SENSITIVE/COLD SENSITIVE PHENOTYPE. R.R.Friis, Institut für Virologie, Fachbereich Humanmedizin, 6300 Giessen W.-Germany.

GI 251, a mutant bearing a single apparent lesion in src produces an interesting phenotype in infected cells: according to parameters of transformation such as disappearance of fibronectin, disorganization of stress fibers, elevated hexose transport, and production of plasminogen activator protease, this mutant is temperature sensitive, the transformed phenotype being expressed at 35 C, and suppressed at 42 C. In terms of transformed growth behaviour, however, this mutant produces a cold sensitive phenotype; mutant-infected cells grown at 35 C behave like normal cells, while sister cultures maintained at 42 C grow to high density independent of a serum or anchorage requirement.

Cells infected with this mutant produce unusually large amounts of $pp60^{src}$ and exhibit also high associated protein kinase activity. Therefore, the hypothesis that super-optimal src gene product function inhibits growth has been tested in the following way: cells were infected simultaneously with 3 different Prague and Schmidt-Ruppin strain Rous sarcoma virus src mutants. Production of high levels of $pp60^{src}$ was verified using immunoprecipitation, and growth control at permissive and nonpermissive temperatures was analyzed. The superinfected cells were found to be somewhat leaky at the nonpermissive temperature, but growth was not impaired at 35 C owing to high $pp60^{src}$ levels; rather these cells exhibited an unusually high cloning efficiency in methyl cellulose suspension culture.

644 ADHESION PLAQUES OF ROUS SARCOMA VIRUS TRANSFORMED CELLS CONTAIN THE SRC GENE PRODUCT, Larry Rohrschneider, and Kathleen Shriver, Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104

The transforming protein of Rous sarcoma virus (RSV) is a 60,000 dalton phosphoprotein termed $pp60^{src}$. This protein has been found in the cytoplasm of RSV transformed cells, at gap junctions between tumor cells, and as a perinuclear spot. In addition to these patterns, we have now found by indirect immunofluorescence using rabbit anti-tumor serum specific for $pp60^{src}$, a speckled pattern of fluorescence on the ventral surface of RSV (strain SR-D) transformed NRK cells (SR-NRK). This pattern, as well as all others, was not seen when normal serum was substituted for anti-tumor serum. Likewise anti-tumor serum did not stain uninfected NRK cells. Interference reflection microscopy has been used to analyze the $pp60^{src}$ fluorescence on the SR-NRK ventral surface. This technique has been used to visualize the adhesion plaques or points at which cells attach to a substratum. Simultaneous immunofluorescence and interference reflection microscopy has demonstrated that each speckle of $pp60^{src}$ specific fluorescence on the ventral surface of the SR-NRK cells corresponds to an adhesion plaque. In addition we have shown that SR-NRK cells could be removed from the glass surface leaving behind the adhesion plaques or "feet" still attached to the glass substrate. These isolated "feet" stain specifically for $pp60^{src}$ by indirect immunofluorescence. The RSV src gene product ($pp60^{src}$) also has been observed in adhesion plaques of SR-D transformed CEC and Balb/c fibroblasts as well as tsLA91 infected rat cells grown at the permissive temperature. When the tsLA91 infected rat cells were maintained at the nonpermissive temperature, $pp60^{src}$ was not observed in the adhesion plaques. Adhesion plaques or cellular "feet" appear to act as focal points of microfilament attachment and our observations suggest that $pp60^{src}$ may affect cellular stress fiber integrity by acting at the adhesion plaques.

645 GENERATION OF SARCOMA AND CARCINOMA INDUCING TYPE C VIRUSES FROM C3H/MuLV IN CELL CULTURE. U.R. Rapp and G.J. Todaro. Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.

A cell culture system has been developed in which the evolution of oncogenic type C virus from endogenous C3H/MuLV was studied (Rapp and Todaro 1978, PNAS 75: 2468; Rapp and Todaro 1979, PNAS in press). The critical steps in this process are: (a) A chronic persistent infection, especially of spontaneously or chemically transformed cells. During this process variant virus forms accumulate. (b) "Conversions" or unmasking of the novel virus forms during a few rounds of acute infection in permissive cells. (c) Selection of cells containing viruses with transforming potential by plating in soft agar. Using this procedure, we have been able to select new rapid leukemia, sarcoma and carcinoma inducing viruses.

The genetic changes that occur during chronic infection include alterations such as acquisition of the XC fusion and GIX antigen markers in the envelope gene region. In addition large substitutions with host cell derived genetic material were observed (Devare et al. 1978, J. Virol. 28: 451). Selection of variant viruses with biologically distinct transforming properties has led to the isolation of nonproducer transformed cells in which the new cell derived transforming functions are stably expressed. The genome structure and the new proteins produced by the transforming viruses in the nonproducer cells will be described.

646 CHARACTERIZATION OF FELINE SARCOMA VIRUS ENCODED POLYPROTEINS WITH POSSIBLE TRANSFORMING FUNCTION, John R. Stephenson,* Wim J.M. Van de Ven** and Fred H. Reynolds, Jr.** *National Cancer Institute and **Frederick Cancer Research Center, Frederick, MD. 21701

Several independent isolates of feline sarcoma virus (FeSV) have been described. Such viruses are apparently derived by genetic recombination between feline leukemia virus (FeLV) genomic RNA and host cellular sequences with transforming potential. Two FeSV isolates, one originally described by Gardner and the second by Snyder and Theilen, have been shown to encode polyproteins of around 115,000 daltons molecular weight. These polyproteins contain FeLV structural components at their amino termini and additional nonstructural components with possible transforming function. By tryptic peptide analysis, Snyder-Theilen FeSV P115 is shown to share three of five [^{35}]-methionine labeled peptides contained within the nonstructural component of Gardner FeSV P115, and absent from FeLV translational products including Pr180$^{gag/pol}$, Pr65^{gag} and Pr82^{env}. Both FeSV encoded polyproteins contain multiple sites of phosphorylation within their nonstructural components and possess associated protein kinase activity. The expression of a highly conserved cellular phosphoprotein with binding affinity for Gardner FeSV P115 in cells of a number of mammalian species is also described. This protein, designated P150, exhibits either intrinsic or associated protein kinase activity and is immunologically and structurally distinct from polyproteins encoded by the Gardner or Snyder-Theilen strains of FeSV.

Picornaviruses/Togaviruses/Hepatitis

647 A HUMAN HEPATOMA CELL LINE CONTAINS HEPATITIS B DNA AND RNA SEQUENCES, Patrick Gray, Jeffrey C. Edman, Pablo Valenzuela, Howard M. Goodman, and William J. Rutter, University of California, San Francisco, San Francisco CA 94143

The Hepatitis B Viral (HBV) genome has been cloned in a bacterial plasmid and used to detect HBV complimentary sequences in cultured human hepatoma cells (Alexander cells). This cell line, originally isolated from a patient with primary liver carcinoma and previous HBV infection (Alexander et al., S. Afr. Med. J. (1976) 50 2124), expresses HBV surface antigen when grown in tissue culture. Radioactively labeled cloned HBV DNA was hybridized to Southern blots of restriction nuclease digested Alexander cell DNA. At least six copies of HBV DNA are integrated into the genomic DNA of Alexander cells. The six integrated HBV DNA sequences are unlinked to each other and are flanked by independant Alexander DNA sequences. Two of the HBV DNA inserts are incomplete segments of the HBV genome. However, all of the inserts contain the HBV surface antigen gene region. Radioactively labeled cloned HBV DNA was also hybridized to Northern blots of Alexander cell RNA. A single RNA species hybridizes to HBV DNA. This RNA is 1700 bases in length and is polyadenylated. The coding region of the HBV surface antigen gene specifically hybridizes to this RNA.

648 CONTROL OF HEPATITIS B SURFACE ANTIGEN AND PLASMA PROTEIN SYNTHESIS IN A HUMAN LIVER-DERIVED CELL LINE, David P. Aden and Barbara B. Knowles, The Wistar Institute, Philadelphia, PA 19104

A hepatoma-derived cell line (Hep 3B) has been established in our laboratory that synthesizes a number of the human plasma proteins normally produced by the parenchymal cells of the liver and in addition alpha-fetoprotein and hepatitis B surface antigen (HBsAg) are synthesized. Supernatants from cultures have been quantitatively analyzed for albumin, alpha-fetoprotein (AFP) and HBsAg during the phases of cell growth. Albumin appears to be constitutively synthesized with the amount in the culture supernatant related to cell number and length of time in contact with the cells. AFP is continuously synthesized but its rate of production increases during late log phase, paralleling HBsAg synthesis. In contrast, HBsAg is not detectable during the lag or early log phases of growth, but first becomes detectable in the supernatant and cells during late log phase. HBsAg in the culture supernatant increases rapidly during this phase and reaches a peak near the end of the culture's growth. Control of HBsAg synthesis will be discussed in terms of cell density, cell contact and regulation through the culture supernatant fluid. The HBsAg produced by the cell lines will be characterized as to polypeptides present. The integration of hepatitis B viral DNA sequences in the cells of this tumor-derived line and somatic cell hybrids between murine cells and this line is under investigation. Results are compared to the PLC/PRF/5 line, which is the only other known line that produces HBsAg.

649 INDUCTION OF HBsAg IN HEPATOMA DERIVED CELL LINES, Gordon R. Dreesman, Paul Oefinger, and David L. Bronson, Baylor College of Medicine, Houston, TX 77030 and University of Minnesota College of Health Sciences, Minneapolis, MN 55455

Two hepatoma cell lines were studied for their ability to produce hepatitis B surface antigen (HBsAg) after induction with dexamethasone (DXM), 5-iodo-2-deoxyuridine (IdUrd), or a combination of the two. The first cell line designated PLC/PRF/5, developed by Dr. J.J. Alexander, actively secretes HBsAg into the tissue culture media. A second hepatoma cell designated Mahlavu, derived by Dr. O.W. Prozesky, was selected as a control because it was reported to be negative for HBsAg production. Treatment of the PLC/PRF/5 cells with DXM for 7 days increased HBsAg production as evidenced by RIA determinations of the supernatant, by three-fold over that observed for supernatants obtained from untreated cultures. Induction with a mixture of IdUrd and DXM resulted in levels of HBsAg production intermediate between that produced by DXM alone versus that of untreated cells. In contrast supernatants of both treated and untreated Mahlavu cells were completely negative for HBsAg. Both cell lines were examined for the presence of intracellular HBsAg production by the peroxidase-anti-peroxidase (PAP) method utilizing specific rabbit anti-HBs. HBsAg was localized in the cytoplasm of the PLC/PRF/5 cells and induction did not increase its relative qualities. Unexpectedly HBsAg was observed in the cytoplasm in rare cells of the Mahlavu line using the sensitive PAP method. After treatment with DXM, HBsAg was observed in 30-40% of the cells. These results suggest that the HBV genome is present in both cell lines which can be induced preferentially with DXM. However, secretion of HBs Ag appears to be blocked in the latter cell line.

650 BIOCHEMICAL STUDIES OF ISOLATED PLASMA MEMBRANES OF SINDBIS VIRUS INFECTED BHK-21 CELLS, Hans Scheefers, Ursula Scheefers-Borchel and Dennis T. Brown, Cell Research Institute, The University of Texas at Austin, Austin, TX 78712

Plasma membranes were prepared in pure form from Sindbis virus infected BHK-21 cells by the method of latex bead injestion. Biochemical analysis of these surface membranes revealed the presence of the precursor glycoprotein PE_2 in the plasma membrane and provided conclusive evidence that the proteolytic processing of this glycoprotein takes place in the plasma membrane. Physical-chemical studies on the association of the Sindbis nucleocapsid with inner surface of the modified plasma membrane are facilitated by the accessibility of the cytoplasmic surface of the latex bead associated membrane.

651 GLYCOSYLATION OF VIRAL GLYCOPROTEINS: ROLE OF VIRAL GENES. Lawrence A. Hunt, Dept. Microbiology, Univ. Kansas Medical Center, Kansas City, Kansas 66103.
Although normal cellular enzymes are apparently responsible for the entire glycosylation of membrane glycoproteins of lipid-enveloped animal viruses, a role for viral genes encoding these membrane polypeptides has been previously demonstrated in comparisons of viral glycopeptides from different viruses replicated in the same host cell type (Sefton, J.Virol. 17: 85, 1976). Examination of viral glycopeptides from Sindbis virus grown in various normal and lectin-resistant cell lines indicates that a virus can acquire carbohydrate structures for its glycoproteins which are unique and not found in significant quantities in the membrane glycoproteins of the uninfected host cell or in the glycoproteins of other viruses grown in the same host cell type. These unusual small neutral oligosaccharides ($Man_3GlcNAc_2$-ASN) presumably result from alterations in the trimming and processing of the common, large oligomannosyl precursor structure in virus-infected cells (Hunt et al., Proc. Natl. Acad. Sci. USA 75:754, 1978). The influence of a viral gene for a non-structural protein on the glycosylation of viral glycoproteins was also examined by high resolution gel filtration and specific glycosidase digestions of glycopeptides from PrC RSV and transformation-defective PrC RSV replicated in the same strain of chicken embryo fibroblasts. The major difference attributable to the presence or absence of the SRC gene product in the infected host cells involves an increase in the amount of larger acidic oligosaccharides relative to the smaller acidic oligosaccharides and neutral oligosaccharides that are characteristic of Sindbis virus grown in the same host cells. (supported in part by PHS grant # AI 14757)

652 Title: THE ROLE OF DEFECTIVE INTERFERING PARTICLES AND VIRAL MUTATIONS IN SINDBIS VIRUS-MEDIATED VIRAL PERSISTENCY. Barbara Weiss, Stephan Monroe and Sondra Schlesinger, Washington University Medical School, St. Louis, MO 63110.
Persistently infected BHK cell cultures were established with wild type Sindbis virus in the presence of defective interfering (DI) particles. These cells have been in culture for over one year and continuously release a small plaque, RNA- temperature sensitive mutant as well as a variable spectrum of DI particles containing many discrete size classes of RNA. The cloned infectious viral mutant can initiate persistency in the absence of detectable DI particles. Prolonged cultivation of these cells, however, results in the appearance of and enrichment for DI particles. The early, DI negative cultures are resistant to superinfection with Sindbis virus, but are susceptible to vescular stomatitis virus (VSV) and Semliki Forest Virus (SFV). When these cells become DI positive they acquire additional resistance to SFV, but not to VSV. This resistance appears to result from heterologous interference by Sindbis DI particles.

Our data support an important role for DI particles in initiating alphavirus persistency with a highly virulent virus. By extending the life of infected cells, they permit the selection of a variant with reduced virulence. This variant alone can reinitiate a carrier state. The evolution of DI particles in cells infected with this mutant may reflect both the ease with which DI particles are generated in BHK cells and a possible selective advantage such dually infected cells would have.

653 CHARACTERIZATION OF THE HYDROPHOBIC TAILS OF THE SINDBIS VIRION GLYCOPROTEINS. Charles M. Rice, Ellen G. Strauss, and James H. Strauss, California Institute of Technology, Pasadena, California 91125.

Digestion of intact Sindbis virions with α-chymotrypsin gives rise to a particle of lighter density containing two protease-resistant fragments of the glycoproteins embedded in the lipid bilayer as well as the intact nucleocapsid. The apparent molecular weights of these fragments are 10,000 and 5,000 daltons. Two-dimensional thin layer as well as high pressure liquid chromatographic techniques for separation of tryptic and/or chymotrypic digests of these fragments have shown that the 10K dalton fragment is derived from E2 (PE2), and the 5K dalton fragment from E1 (PE1). In a two-phase, aqueous-organic system at neutral pH, PE1 is much more soluble (~4-fold) in the organic phase than is PE2. Both fragments are rich in hydrophobic amino acids, but PE1 has a larger proportion of hydrophobic residues than PE2. Other workers have shown that when Sindbis is grown in the presence of [^{3}H] palmitate, both E1 and E2 are labeled. Digestion of [^{3}H] palmitate labeled virions with α-chymotrypsin gives rise to labeled material which co-migrates with PE1 and PE2. The percentage of [^{3}H] palmitate recovered in these fragments accounts for most, if not all, of the glycoprotein associated label. These results suggest that the covalently attached palmitate molecules are clustered in hydrophobic, membrane-associated regions of the glycoproteins.

654 IN VITRO SELECTION OF AN ATTENUATED VARIANT OF SINDBIS VIRUS, R. S. Baric, D. B. Moore, and R. E. Johnston, North Carolina State University, Raleigh, N.C. 27650.
Attenuated virus strains have been derived by an empirical process guided by the observation that blind serial passage in heterologous tissue culture selectively enriches for mutants with decreased pathogenicity for the animal host. We have superimposed a selective pressure for rapid growth upon this process. Sindbis virus (SB) was passaged serially in BHK cells by harvesting only the virus released within the first two hours following the end of the latent period. An attenuated variant (SB-RL) was cloned from the eleventh such passage. One hundred percent mortality was observed in litters of one day old mice injected s.c. with 25 or more PFU of SB. In SB-RL infected litters, the survival rate decreased from 60 to 30% with doses ranging from 2.5 to 2.5×10^6 PFU/mouse. The mean day of death in SB-RL infected litters decreased with increasing dose, while this parameter in SB infected mice was independent of dose. SB-RL was found to protect mice from challenge with SB. In tissue culture, SB-RL was characterized by a significant reduction in latent period and an increased rate of penetration. These characteristics were expressed only in the BHK cell line used for the original selection, but not in other cell lines. SB-RL also produced higher virus titers and exhibited an increased rate of RNA synthesis. However, the expression of these characteristics was independent of cell type. SB-RL produced small plaques in all cell lines tested but was not temperature sensitive. SB-RL could be distinguished from SB on the basis of antibody neutralization, suggesting an alteration in the E2 glycoprotein. Moreover, our preliminary results indicated that SB-RL produced defective-interfering particles more rapidly than SB.

655 A NOVEL ANTIVIRAL ACTIVITY PRODUCED BY MOSQUITO (AEDES ALBOPICTUS) CELLS PERSISTENTLY INFECTED WITH SINDBIS VIRUS, Ursula Scheefers-Borchel, Birgit Riedel, Hans Scheefers and Dennis T. Brown, Cell Research Institute, The University of Texas at Austin, Austin, TX 78712
Mosquito (Aedes albopictus) cells persistently infected with Sindbis virus release a dialysable factor into the medium which is capable of specifically reducing the yields of Sindbis virus during the "acute" phase of infection by 2 logarithms when applied to mosquito cells prior to infection. Mosquito cells pretreated with this "factor" do not show the characteristics of the acute phase of infection but rather demonstrate directly the characteristics of persistent infection and produce only low levels of progeny virus. The antiviral activity is inactivated by protease treatment and heat (56^0). It differs from interferon produced by vertebrate cells in that it is cell specific as well as virus specific. The action of this novel antiviral activity and its molecular basis will be discussed.

656 PERSISTENT INFECTION OF SEMLIKI FOREST VIRUS IN MOUSE L929 CELLS, Judy L. Meinkoth and S.I.T. Kennedy, University of California, San Diego, La Jolla, California 92093
A persistent infection of mouse L929 cells using the alphavirus Semliki Forest virus (SFV) could only be established using an inoculum rich in defective interfering (DI) particles. Once established, such a carrier culture is characterized by cells which are usually morphologically identical to uninfected cells, but release variable titers of infectious virus and periodically enter a state of crisis during which many of the cells perish. After the first crisis, DI particles could not be detected at any time in the carrier culture. Only a small proportion of the persistently infected cells release infectious virus and are positive for viral structural antigen. Interferon is present in variable quantities in the fluid from the carrier culture. Using oligonucleotide fingerprinting we have demonstrated that viral genomic mutations occur during persistence and that many of these mutations occur in genes coding for the virus structural proteins. Mutations in the structural genes might affect several properties of the virus including cell recognition, morphogenesis, regulation of intracellular virus specific RNA synthesis and antigenic determinants. Using monoclonal antibodies to the virus structural proteins studies are underway to determine the possible phenotypic consequences of the continuing mutations in the viral genome.

657 INTRACELLULAR EVENTS IN CLONED POPULATIONS OF CELLS PERSISTENTLY INFECTED WITH JAPANESE ENCEPHALITIS VIRUS. Connie S. Schmaljohn and Carol D. Blair, Colorado State University, Fort Collins, Colorado 80521

Cell clones obtained from two lines of cultured cells persistently infected with Japanese encephalitis virus (JEV) initially neither expressed viral antigen nor released infectious virus but retained viral RNA. Upon superinfection with wt JEV, clones became indistinguishable from parent persistent infections (PI): i.e. showed no viral CPE, expressed viral antigen in most cells and released low levels of infectious virus which suggested replication interference. Superinfection of clones with a temperature sensitive (ts) mutant of JEV at the permissive temperature resulted in production only of ts mutant JEV indicating no activation of latent virus. Continued passage of clones resulted in spontaneous release of infectious virus from some non-superinfected clones. Superinfected non-releaser or spontaneous releaser clones examined by EM showed little evidence of viral replication even when all cells expressed viral antigen. Intracellular virus-specific RNA patterns of non-releaser clones indicated that only the two small subgenomic RNAs (12 and 15S) were produced in large amounts while spontaneous releasers and superinfected clones produced genome size (40S) RNA as well but in lesser amounts than wt JEV control infections. The RNA patterns observed are similar to the early events in wt JEV replication and suggest that interference with virus replication occurs at the level of genome-length RNA synthesis.

658 STUDIES ON THE GENETIC BASIS OF VIRAL ATTENUATION USING A TEMPERATURE-SENSITIVE, ATTENUATED MUTANT OF VENEZUELAN ENCEPHALITIS VIRUS, Emilio A. Emini and Michael E. Wiebe, Dept. of Microbiology, Cornell Univ. Medical College, N.Y., N.Y. 10021

A temperature-sensitive mutant, ts 126, derived from the hamster-virulent 68U201 wild-type (wt) strain of Venezuelan encephalitis virus, an alphavirus, has been shown to be attenuated. Although it is temperature-sensitive, the mutant replicates in infected hamsters and elicits production of protective antibodies (Krieger, J.N. et al., 1979, Infect. Immun. 25: 873-879).

We describe here further phenotypic differences between ts 126 and 68U201 wt in an attempt to localize the genetic basis of the mutant's attenuated virulence. ts 126 was shown to differ from the parent virus with respect to virion structure-dependent, particularly surface structure-dependent, characteristics: temperature lability, plaque sizes in Vero cells, and binding properties to hydroxylapatite. The common genetic basis of all these phenotypic differences was demonstrated by the isolation of a number of independently-arising, stable genetic revertants of ts 126. These revertants exhibit characteristics identical in every respect to 68U201 wt.

The nature of the phenotypic differences between the parent virus and ts 126 indicates that the genetic lesion responsible for the loss of virulence by ts 126 is most probably located in the structural gene(s) coding for one of the two virion surface glycoproteins.

659 THE STRUCTURAL PROTEINS OF RUBELLA VIRUS, Diane Van Alstyne, and Ehleen M. Bohn, Department of Pediatrics, University of British Columbia, Vancouver, B.C., Canada.

Rubella virus has been purified using 25-45% discontinuous and 30-45% continuous renografin gradients. The virions were dissociated and iodinated with ^{125}I. The labeled proteins were then subjected to polyacrylamide slab gel electrophoresis and autoradiography. Four structural proteins were observed with molecular weights of 44,000, 41,000, 24,000 and 19,000 daltons, and a total molecular weight of 128,000 daltons. These data are not in close agreement with previous reports which have employed different purification and labeling technics. However, the improved purification and the increased sensitivity afforded by the technics described here provide a more accurate analysis of the structural proteins of Rubella virus than has been possible previously.

Rubella virus has hemagglutination properties like those of alphaviruses but shows no serological cross-reactivity with other togaviruses. Although Rubella is not arthropod-borne, it has been placed in the Togaviridae as an ungrouped member of the family based mainly on its morphological and biological similarities to the accepted togaviruses. The data presented here compare favorably with the molecular weights of the structural proteins of Sindbis virus and Semliki Forest virus and provide additional biochemical evidence in support of the classification of Rubella virus in the family Togaviridae.

660 THE PURIFICATION OF RUBELLA VIRUS, Ehleen M. Bohn and Diane Van Alstyne, Department of Pediatrics, University of British Columbia, Vancouver, B.C., Canada.

Rubella virus has been propagated in murine fibroblasts (L cells) and purified using two renografin gradients. The virus was grown in the presence of 2 μCi/ml ^{3}H-uridine, pelleted from tissue culture media 5 days post-infection and applied to a 25-45% discontinuous gradient. A single, sharp band was observed at the interface. This band was collected and applied to a 30-45% continuous gradient which separated intact labeled virus from a large amount of ^{3}H-labeled, light density material. Sucrose gradients did not permit this degree of separation.

Difficulties were encountered in the course of this study in obtaining consistently good yields of infectious virus from tissue culture. The yield appeared to depend on both the viral stock and the type of cell culture employed. High yields of infectious virus (greater than 10^{7} PFU/ml) were never obtained. It was also difficult to demonstrate consistent plaque formation with any cell culture tested. Therefore, hemagglutination (HA) and enzyme-linked immunosorbent assays (ELISA) and direct microscopic examination were employed to detect the virus during purification. The ^{3}H-labeled light density peak contained only ELISA activity and no intact virions were found upon microscopic examination. A second ^{3}H-labeled peak with a density of 1.19 gm/cm^{3} demonstrated both HA and ELISA activities and upon microscopic examination was found to consist primarily of Rubella virus.

661 SUBGENOMIC VIRUS-SPECIFIC RNA'S IN FLAVIVIRUS-INFECTED CELLS. Carol D. Blair and Connie S. Schmaljohn, Colorado State University, Fort Collins, Co. 80523.

Evidence has recently been presented that the 40S virion RNA of flaviviruses is the only detectable single-stranded intracellular virus-specific RNA and that it serves as the sole messenger during replication. Data to support these proposals were acquired in studies conducted after the end of the eclipse phase of viral growth (16-24 hr PI). We have examined early eclipse phase (3-6 hr PI) Japanese encephalitis virus-specific RNA and have observed four sedimentation classes: 40S, 30S, 22S, and 15S. The previously undetected 15S species was most prominent at 3 hr PI, and continued to be synthesized in small amounts throughout infection. Upon gel electrophoresis, it separated into two distinct single-stranded moities, both of which were mRNA-like in chemical properties and intracellular location. The 22S species was partially double-stranded and probably a replicative intermediate. The 40S RNA became predominant late in infection. After denaturation the 40S and 30S species had identical electrophoretic mobilities and were therefore considered conformational variants of a single molecular class. We propose that flaviviruses synthesize subgenomic mRNAs which are required early in replication and may code for non-structural proteins.

662 SEQUENCE ANALYSIS OF THE POLIOVIRUS GENOME AND MAPPING OF THE GENOME-LINKED PROTEIN, Naomi Kitamura, Cheryl Adler and Eckard Wimmer, S.U.N.Y. at Stony Brook, N.Y. 11794

We are in the process to sequence the poliovirus genome by an adaptation of Sanger's dideoxymethod. This involves reverse transcription of virion RNA, followed by E. coli Pol I catalysed DNA synthesis in the presence of cDNA, dNTP, ddNTP and [5'-^{32}P] labelled RNase T1 or RNase A resistant oligonucleotides of virion RNA as primers. So far, we have sequenced segments of the RNA totaling 5,800 nucleotides. A sequence of 800 bases from the 3' end reveals that at least 562 nucleotides preceding the poly(A) remain non-translated. A base sequence in the 3'-terminal half of the genome corresponding to known amino acid sequences of the genome-linked protein (VPg) has been detected. These data suggest that VPg is part of the viral polypeptide NCVP1b. Thus, VPg and the viral RNA polymerase NCVP4 originate from the same polypeptide precursor. VPg is, at the most, 27 amino acids long (max. mol. wt. 3,296). Knowledge of the nucleotide sequence of virion RNA will allow us to accurately determine the extent of deletions in defective interfering particles (deletion mutants) of poliovirus.

663 RIBOSOME BINDING TO THREE SITES ON POLIO GENOMIC RNA, Kenneth McClain, Margaret Stewart, Robert Crouch, Jacob Maizel, Jr., National Institutes of Health, Bethesda, MD. 20205

Rabbit reticulocyte ribosomes were bound to genomic polio RNA in vitro. Unprotected RNA was digested with T_1 RNase and the ribosomes with protected oligonucleotides were isolated on a glycerol gradient. After deproteinization, the oligonucleotides were analyzed by two dimensional polyacrylamide gel electrophoresis. Three separate oligonucleotides were found to be protected by ribosomes whereas other fragments were protected by non-ribosomal proteins. Secondary digests by T_1 and pancreatic RNase followed by two dimensional gel electrophoresis demonstrated that each of these three ribosome protected oligonucleotides was a distinct specie. In another experiment, subgenomic fragments of polio RNA were generated by digestion with RNase III and poly A-containing fragments selected by poly U sepharose affinity chromatography. In vitro translation of different size classes of fragments showed that proteins could be made from two oligonucleotides which were approximately 0.55 and 0.92 map units from the 5' end, as well as from the full length genome. Corroborative evidence for ribosome binding at three sites was obtained by electron microscope studies.

664 TWO-DIMENSIONAL ANALYSIS OF ENCEPHALOMYOCARDITIS VIRUS, Margaret A. Churchill and Roger J. Radloff, University of New Mexico School of Medicine, Albuquerque, N.M. 87131

We have examined encephalomyocarditis virus-specific proteins from purified virus and infected cells by two-dimensional polyacrylamide gel electrophoresis. This method combines isoelectric focusing in the first dimension with sodium dodecylsulfate geleelectrophoresis in the second dimension. Two of the four capsid proteins of purified virus, α and β, could be resolved in this system. We have recently been able to resolve a third capsid protein, γ, and ε, a precursor protein to the capsid proteins β and δ with nonequilibrium pH gradient electrophoresis. Capsid proteins α, β, and ε were resolved from infected cell extracts and their estimated isoelectric points were 7.2, 6.6, and 5.9, respectively. Noncapsid proteins E, F, H, and I were also resolved. Noncapsid protein H was separated into two polypeptides of slightly different molecular weight (H and H1). The isoelectric points of the noncapsid proteins were estimated: E, 7.1; F, 7.7; H, 5.7; H1, 5.4; and I, 5.4. We have begun to study the isoelectric focusing characteristics of the capsid and noncapsid proteins of several EMC virus temperature sensitive mutants.

665 TEMPERATURE SENSITIVE MUTANTS PRODUCED IN HUMAN LYMPHOID CULTURES PERSISTENTLY INFECTED WITH POLIOVIRUS, Richard I. Carp, New York State Institute for Basic Research in Mental Retardation, Staten Island, N. Y. 10314

Persistent infections of human lymphoid cells with attenuated and virulent polioviruses can readily be established. Infected and control cultures were passaged by 1-2 dilutions at weekly intervals. Virus was present at titers ranging from 10^4 to 10^6 plaque forming units/ml throughout incubation periods as long as 43 weeks. The growth of virus infected and control cultures was similar. Light microscopic examination failed to show any differences between infected and control cultures and the proportion of live cells was similar. Plaque purified virus populations were prepared from virus present in cultures infected with attenuated virus 67 days previously. For the virus used as inoculum and for 8 of 8 plaque purified populations derived from this virus pool, titers at 33°C and 37°C were similar. The same was true for 10 of 12 plaque populations derived from virus in the 67 day lymphoid cultures. For the other 2 plaque pools, the titers at 33°C were at least 2 $\log_{10}$ units higher than at 37°C. The results with virulent virus were more striking. For virus used as inoculum and for plaque populations derived from that pool the titers at 40°C and at 37°C were similar. In contrast, approximately 50% of the purified plaque populations derived from lymphoid cultures (from 23 to 133 days after infection) gave titers at least 1.5 $\log_{10}$ units higher at 37°C than at 40°C.

666 CHARACTERISTICS OF A VARIANT OF LACTATE DEHYDROGENASE VIRUS (LDV), Margo A. Brinton Wistar Institute, Philadelphia, PA 19104 and Douglas Martinez, Merck Institute for Therapeutic Research, West Point, PA 19486

LDV replicates rapidly in all strains of mice so far tested producing a persistent infection. A relatively high level of infectivity is continually present in the blood of infected mice, but no disease symptoms are observed. Recently,we have isolated a variant of LDV which was associated with an Ib leukemia cell line that had been passaged in C58 mice for about 20 years. This LDV strain (C58-LDV) is able to produce a polioencephalomyelitis in C58 mice. Unique characteristics of both the host and the virus strain are required for the production of this paralysis. LDV's have been previously isolated in a number of laboratories, but no technical methods existed for distinguishing differences between these isolates. The assessment of the ability to induce paralysis in C58 mice and the measurement of antigenic crossreactivity by RIA have provided initial means for characterization. Persistently infected mice circulate virus complexed with antiviral antibody. These complexes are infectious. We have found that challenge of persistently infected mice with homologous virus results in an increase in the level of antiviral antibody. The C58-LDV appears to be more immunogenic than other LDV's, since it elicits a 40-fold increase in antibody as compared to a 5-fold increase elicited by other LDV's. We have recently observed that C58-LDV is capable of replicating in neurons as assessed by EM analysis of spinal cord lesions in C58 mice and by infection of CNS cells in culture. Macrophage-like cells had been thought to be the only cells replicating LDV. Levels of infectivity and antiviral Ab in the CSF are being evaluated to determine the abilities of the various LDV strains to enter the CNS of C58 and control mice.

667 SCRAPIE--AN EXPLANATION FOR ITS EXTREME RESISTANCE TO RADIATION, Robert G. Rohwer, D. Carleton Gajdusek, National Institutes of Health, Bethesda, Md. 20205

The infectious agent of scrapie disease is two times more resistant to inactivation by ionizing radiation than are the most resistant viruses yet characterized in this way. Computation of its radiation target size using conventional assumptions suggests a subviral nucleic acid size of only 60,000 to 150,000 daltons. Values at least this small are indicated for the related human diseases, kuru and Creutzfeldt-Jakob Disease. These small target sizes have led to the suggestion that these diseases are caused by viroids or even novel mechanisms which do not involve nucleic acids. However, unlike the viroid agents, scrapie infectivity is very sensitive to phenol extraction. Also, other physical methods such as filtration, sedimentation and bouyant density suggest virus-like properties for the infectious agent.

One solution to this paradox is a model in which the titratable infectious unit of these diseases exists as an aggregate of several competent viruse particles, all of which must be inactivated before the infectious unit itself is inactivated. Depending upon the size distribution of these presumed aggregates, the inactivation curve observed for the scrapie agent can be fit exactly by conventionally sized viruses.

Ample qualatative evidence for aggregation of the sort required in this model exists in the scrapie literature. In an attempt to quantitate this phenomenon, we have determined by means of sonication, a minimum mean aggregate size of 20 particles/aggregate in suspensions of scrapie mouse brain.

Adenoviruses/SV40/Polyoma I

668 CORRELATED EXPRESSION OF SV40 NUCLEAR T-ANTIGEN AND OF SV40 T-ANTIGEN RELATED SURFACE ANTIGEN IN CELLS TRANSFORMED BY SV40 WILD TYPE AND AN SV40 tsA MUTANT, Wolfgang Deppert, Jutta Lange-Mutschler and Roland Henning, University of Ulm, F.R.G.

We studied the postulated involvement of SV40 T-antigen (T-Ag) in the formation of SV40 tumor specific transplantation antigen (TSTA). T-Ag related surface antigens were detected on living SV40 transformed cells as well as on cells infected with adeno2-SV40-hybrid virus by immunofluorescence microscopy and by an ^{125}I-protein A binding assay. The sensitivity of both methods could be increased by careful fixation of the cells with formaldehyde at 0° C. Upon permeabilization of formalin fixed cells, in addition to T-Ag related surface antigen nuclear T-Ag could be visualized by a double staining technique in all the SV40 transformed cell lines analyzed. In rat cells transformed by the temperature-sensitive SV40 A-gene mutant tsA 28.3 both antigens were detectable at the permissive temperature. Upon shift to the non-permissive temperature the expression of both antigens decreased simultaneously. In these cells the expression of both SV40 T-Ag related surface antigen and nuclear T-Ag was strongly correlated with the synthesis of the T-Ag polypeptide, suggesting that both nuclear T-Ag and T-Ag related surface antigen are products of the SV40 A-gene. Altogether, these data indicate that T-Ag related antigens are present on the surface of SV40 transformed cells and might participate in the formation of SV40 TSTA.

669 PHOSPHORYLATION SITES OF SV40 T ANTIGEN,

Karl Heinz Scheidtmann, Arlene Carbone and Gernot Walter,
Institut für Immunbiologie, Universität Freiburg,
Stefan-Meier-Str.8, West Germany.
SV40 large T antigen was shown to be a phosphoprotein(1). On the average, each T antigen molecule contains four phosphates one of which is bound to threonine and the others to serine residues(2).
The locations of the phosphoaminoacids within the polypeptide chain were determined by differential labeling with various aminoacids and ^{32}P and subsequent fingerprinting. The metabolism of the different phosphorylresidues is under investigation.

(1) Tegtmeyer,P. et.al., 1977, J.Virol. 21, 647-657

(2) Walter,G. and Flory,P.J., 1979, Cold Spring Harbor Symposium on Viral Oncogenes, in press.

670 TWO FORMS OF SV40 LARGE-T ANTIGEN IN TRANSFORMED CELLS, Ed Harlow and Frank McCormick, Imperial Cancer Research Fund, London, England

Serum raised against a 53K murine phosphoprotein precipitates both the 53K imunogen and SV40 large-T from transformed cells. This serum, designated F5, has no detectable activity against large-T, but precipitates both phosphoproteins because 53K and large-T form a stable complex within the nucleus of transformed cells. No other proteins can be detected in the complex. All the 53K recognized by F5 is present in a high molecular weight complex(600,000-1,000,000 daltons), but large-T is found in two separate species. The complexed form of large-T is more highly phosphorylated than the free form, and during a pulse label, two moles of inorganic phosphate are incorporated into the complexed large-T for every one mole incorporated into free large-T. Analogous complexes have been found in all SV40 transformed cells that have been tested to date.

671 CELLULAR PROTEINS ASSOCIATED WITH SV40 t-ANTIGEN, Kathleen Rundell, Northwestern University Medical School, Chicago, Ill. 60611

The SV40 t-antigen is able to form a complex with two cellular proteins (56K and 32K). The apparent molecular weights of the cellular proteins were determined on 20% polyacrylamide SDS gels, but vary widely with different SDS gel systems. A similar complex is formed between the BKV-virus t-antigen and the same two cellular proteins. The cellular proteins are present in immunoprecipitates of radiolabeled cell extracts only when viral 17K protein is available to allow their co-precipitation, but are not directly recognized by antisera raised in tumor bearing hamsters. Identification of the proteins from radiolabeled uninfected cells requires the addition of unlabeled crude extracts or partially purified preparations that contain the SV40 17K protein. The cellular proteins are not induced by SV40 infection, and are synthesized both in serum-starved and in theophylline-blocked uninfected cell populations.

672 SV40 TUMOR ANTIGEN: DOMINANCE OF CYTOPLASMIC MUTANT PHENOTYPE OVER WILD-TYPE NUCLEAR LOCALIZATION, Robert E. Lanford and Janet S. Butel, Baylor College of Medicine, Houston, TX 77030

A mutant of the defective SV40-adenovirus 7 hybrid, PARA, induces the synthesis of SV40 large T-antigen (T-ag) that is not transported to the nucleus and accumulates in the cytoplasm of infected or transformed cells. The effect of this cT mutation on the transport of wild-type (WT) T-ag was examined by co-infection experiments. Immunofluorescence staining for T-ag revealed that when SV40-transformed green monkey kidney cells were infected with the cT mutant of PARA {PARA(cT)} there was a total loss of nuclear T-ag (nT-ag) reactivity concomitant with the appearance of cytoplasmic T-ag (cT-ag) reactivity. This suggested that the cT-ag phenotype was dominant over the nT-ag phenotype. The dominance of the cT-ag phenotype was also manifest in cells co-infected with PARA(cT) and either SV40 or WT PARA, both of which induce nT-ag during single infection. Two lines of evidence indicate that the absence of nuclear T-ag reactivity in co-infected cells is due to a failure of migration of WT T-ag into the nucleus, rather than an inhibition of its synthesis. First, co-infection of cells with SV40 and either helper adenovirus or PARA-adenovirus populations, at the multiplicities of infection employed in these studies, did not reduce the yields of infectious SV40. Second, cells co-infected with PARA(cT) and deletion mutants of SV40 which encode T-ag polypeptides of reduced molecular weight expressed the cT-ag phenotype, and the presence of the deleted forms of T-ag was confirmed by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. The dominant effect of the cT mutation was specific for transport of SV40 T-ag, since normal migration of adenovirus tumor and virion antigens as well as SV40 virion antigen occurred.

673 MUTANTS AFFECTING MEDIUM T ANTIGEN OF POLYOMA VIRUS, D.J. Templeton and Walter Eckhart, Salk Institute, La Jolla, CA 92112.

The early region of polyoma virus encodes three tumor antigens of molecular weights 88K, 51K, and 25K daltons. The coding regions for the three T antigens have been analyzed by comparing the tryptic peptides of the isolated proteins to the nucleotide sequence of polyoma DNA. A protein kinase activity, having the unusual specificity of phosphorylating tyrosine, is associated with polyoma T antigen immunoprecipitates. We are studying the effect of mutations in the coding region for the medium T antigen on infection, cell transformation, and tyrosine phosphorylation. A deletion mutant, kindly provided by Mary Bendig and Bill Folk, synthesizes a medium T antigen of altered mobility. This medium T antigen, like its wild type counterpart, is phosphorylated on tyrosine in vitro. We are attempting to isolate and characterize other mutants in this region.

674 HR-T MUTANTS OF POLYOMA VIRUS, Gordon G. Carmichael and Thomas L. Benjamin, Harvard Medical School, Boston, MA 02115

We have determined the DNA sequence alterations in non-transforming polyoma hr-t mutants. Nineteen such mutants have been isolated in our lab. The lesion in each of these mutants lies in the proximal early region of the viral genome, between 79 and 85 map units. Sixteen of these mutants carry deletions in Hpa II fragment 4 and fail to induce the appearance of normal small (22K) and middle (36K, 56K and 63K) T antigens. Of seven which have been sequenced, six are frameshift mutants; the exception carries an in-phase deletion and makes an appropriately shortened middle T polypeptide. The remaining three mutants make normal amounts of a 56K middle T and reduced amounts of a 22K small T; they do not induce the appearance of the 36K and 63K species and the mutant 56K antigen is inert in an in vitro protein kinase assay while the wt antigen can be highly labeled with ^{32}P. Each has the same alteration in the DNA at map position 84: an insertion of an ATA (ile) codon in the small T/middle T coding region, followed by a G to A transition (asp to asn). Thus, the non-deletion hr-t mutants code for small and middle T antigens which differ from the wild type polypeptides by only two amino acids. We shall discuss ongoing experiments designed to assess the individual functions of the various polyoma T antigen species in productive infection, in the alteration of cell morphology and growth properties, and in neoplastic transformation.

675 ANALYSIS OF CLONES TRANSFORMED BY COMPLEMENTATION BETWEEN HR-T AND TS-A MUTANTS OF POLYOMA VIRUS. Michele M. Fluck* and Thomas L. Benjamin, Department of Pathology, Harvard Medical School, 25 Shattuck Street, Boston, Mass. 02115

Stably transformed clonal cell lines have been obtained in mixed infections with transformation defective mutants of polyoma virus, hr-t and ts-a. Information on the nature of the viral genome(s) and viral function(s) retained and expressed in transformed "complementation clones" is relevant to the question of the role of the hr-t and ts-a genes in the initiation and maintenance of transformation, the assumption being that the expression of the viral maintenance function - but not necessarily of the initiation function - must persist in transformed cells.

Sixty-five clones of rat cells (Fisher F-111 and NRK) obtained by mixed infections between hr-t (NG-18, II-5, SD-15) and ts-a (ts-25D, ts-616, ts-48, ts-a) mutants have been studied in terms of the T-antigens expressed and the nature of the virus obtained by fusion with permissive cells or revealed by Southern blotting. Viral proteins are expressed both from the hr-t and ts-a alleles. All clones analyzed express middle and little t-antigens in both Fisher and NRK clones. Thirty percent of NRK and about 10% of the Fisher clones have either no or a thermolabile expression of large T-antigen. Virus can be rescued from the majority of the Fisher clones and from a minority of the NRK clones. Both hr-t and ts-a alleles are rescued. In addition, wild type recombinants are observed in the majority of the Fisher rat clones. We are testing the hypothesis that wild type genomes arise by packaging DNA from concatemers containing hr-t and ts-a alleles in tandem configuration.

*Present address: Microbiology, Michigan State U., East Lansing, MI 48824

676 ALTERED PATTERNS OF INTEGRATED SV40 DNA IN CLONAL DERIVATIVES OF tsA MUTANT TRANSFORMED MOUSE CELLS, John Hiscott, David Murphy and Vittorio Defendi, Department of Pathology, New York University, New York, NY 10016

The SV40 tsA58 transformed mouse clone A21 displays a temperature sensitive transformed phenotype, but colonies can be obtained in soft agar at 40°C with a frequency of less than 1%. Clonal derivatives of A21, isolated by growth in soft agar at 40°C, were analyzed by restriction endonuclease cleavage and Southern blot hybridization. In 10 of 12 clones examined, the conversion of A21 cells to anchorage independent growth at 40°C is accompanied by the acquisition of new sites of integrated SV40. By using the Bgl II endonuclease which lacks specificity for SV40, it was shown that the parental A21 clone contains SV40 sequences in 9 kb and 15 kb fragments of cellular DNA. The clonal derivatives generally retain the 9 kb and 15 kb integration sites but possess additional SV40 insertions within Bgl II fragments of 15 to 25 kb. The viral DNA monomer in both the 9 kb and 15 kb Bgl II inserts is a deletion fragment of 3.8 kb which is arranged in a tandem head to tail fashion in the cellular DNA. These results suggest that the change in biological expression of the transformed phenotype may be accompanied by the rearrangement of integrated SV40 specific sequences.

677 ANCHORAGE-INDEPENDENT GROWTH OF BOVINE PAPILLOMA VIRUS TRANSFORMED HAMSTER EMBRYO CELLS, D.M. Morgan and W. Meinke, University of Arizona, Tucson, Arizona 85724

Cell transformation with bovine papilloma virus (BPV) and BPV DNA is described. Primary hamster embryo cells were obtained by trypsinization of minced embryonic tissue from Golden Syrian hamsters (Graffi or LVG strains) and cell cultures established. Confluent cell cultures were treated with bromodeoxyuridine for about 24 hours prior to infection with BPV or treatment with calcium phosphate-precipitated BPV DNA. Other cells were mock-infected with plain Dulbecco's medium to serve as controls. Infected cells underwent striking morphological changes during the next few weeks; long, spindle-shaped cells became predominant. These cells grew in patterns resembling foci, thus they were tested for anchorage-indendent growth in dilute agarose containing medium. Colonies of these cells became evident in 5-6 weeks. Colonies were removed from the agarose and transferred to dishes of liquid medium. Clones of the morphologically altered cells have been established. This is the first report of BPV-transformed cells demonstrating anchorage-independent growth in agarose and the establishment of cell clones.

678 UNIQUE MECHANISM OF BKV INDUCED TRANSFORMATION AND PERSISTENT INFECTION OF HUMAN FETAL BRAIN CELLS, Hawley K. Linke, George C. Fareed, and Kenneth K. Takemoto, University of California, Los Angeles, CA 90024 and NIAID, NIH, Bethesda, MD 20014

The papovavirus BKV belongs to a group of highly homologous tumor viruses including SV40, Py, and JCV. Like JCV, BKV is of human origin. Despite greater than 80% DNA sequence homology in vital regions of the genomes, the four viruses exhibit extensive divergence in host cell preferences, transforming ability and mechanisms, and (in the case of the human isolates) pathogenicity. We have been examining these processes as regulated by the BKV genome in persistently infected and malignantly transformed human fetal brain cells (BKHFB cells). Our results have indicated that the viral genome as well as the transformed phenotype is maintained by a mechanism other than those demonstrable in either SV40 or Py transformed models or in BKV transformed cells of non-human-neural origin. As with certain hosts of SV40, BKV transformation of HFB cells leads to the induction of presumably cell cycle related, host encoded proteins. Furthermore, the induction of host and viral encoded gene products which may participate in the transformation is regulated by factors outside the cell, such as those factors which may be active in vivo. Data will be presented which identifies both host and viral products and their involvement in the processes of transformation and persistent infection.

679 ANALYSIS OF ADENOVIRUS 2 INDUCED DNA SYNTHESIS IN A ts MUTANT OF THE CELL CYCLE, Mara Rossini, Gerald Jonak and Renato Baserga, Temple University, Philadelphia, Pa. 19140.
tsAF8 cells are temperature sensitive mutants derived from BHK cells that arrest in the G_1 phase of the cell cycle at the nonpermissive temperature of 40.6°C, while growing normally at 32°-34°C. When made quiescent by serum deprivation and subsequently stimulated by addition of fresh medium containing 10% serum, ts AF8 cells do not enter DNA synthesis if incubated at 40.6°C. Infection of quiescent tsAF8 with Adenovirus 2 results in an induction of DNA synthesis either at the permissive temperature or at the nonpermissive temperature. At the permissive temperature viral and cellular DNA are synthesized while at the nonpermissive temperature only cellular DNA is synthesized. Infection of these cells with deletion (Ad5d1312, Ad5d1313) and temperature sensitive (H5ts125 and H5ts36) mutants of Adenovirus indicate that the expression of the early regions 1a and 2 of Adenovirus genome is needed to induce quiescent ts AF8 cells to enter S phase at the permissive temperature, while to overcome the ts block, additional early viral functions are required, such as the expression of region 1b and 5. Microinjection of Ad2 DNA restriction fragments into quiescent ts AF8 cells and their ability to induce DNA synthesis will be discussed.

680 CONSTRUCTION OF COTRANSFORMATION OF HUMAN CELL LINES WHICH EXPRESS SEGMENTS OF THE ADENOVIRUS GENOME
Daniel F. Klessig, Michael R. Wigler and Terri Grodzicker, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Using cotransformation with the HSV-1 thymidine kinase (tk) gene, we have constructed several cell lines that contain and express different segments of the genome of adenovirus type 2 (Ad2). Five lines were derived from the human tk^- 143 cell line (gift of C. Croce) which are both tk^+ and support the growth of an adenovirus type 5 deletion mutant (d1312) that lacks the DNA sequences located between coordinates 1 and 4.5. Although five lines maintain the Ad2 sequences, their expression is unstable. Two lines lost their ability to complement d1312 after several generations even while maintained under selective pressure for tk expression. The remaining three lines continue to support d1312's growth after more than 150 generations although some clones derived from them show different levels of complementation. The viral DNA present in these cells and its transcription will be discussed as will attempts to construct cells expressing other regions of the viral genome.

Adenoviruses/SV40/Polyoma II

681 EXPRESSION OF THE RAT INSULIN GENE CLONED IN SV40, M.Chi Nguyen-Huu, Steve Clark, Paul Berg, and Howard M. Goodman, University of California, San Francisco, CA 94143, Stanford University, Stanford CA 94305
A 1.5 kilobase DNA fragment carrying the rat insulin I gene, including its intervening and adjacent sequences, has been cloned in the late gene region of the monkey virus SV40. Two types of hybrid viruses have been constructed, with the rat insulin gene inserted either in the same orientation as the SV40 late protein coding sequence, or in the inverted orientation. Thus in the latter case the expression of the rat insulin gene should be under the control of its own transcription promoter whereas in the former case transcription could occur from both the viral late region promoter and the rat insulin promoter. These possibilities are being studied by the analysis of rat insulin RNA and protein synthesized in monkey kidney cells infected with a helper phage and either type of hybrid virus.

682 SV40 VECTOR DESIGNED FOR INSERTION OF CODING SEQUENCES UNDER CONTROL OF THE EARLY PROMOTER, Bruce H. Howard and Paul Berg, Stanford University, Stanford, Cal. 94305

Our interest in developing SV40 derivatives as vectors for eukaryotic cells has led to the construction of the recombinant vector pSV2. pSV2 is a hybrid between PBR322 (a 2.3 Kb segment containing the origin of replication and the amp^R gene) and a modified SV40 early region. Foreign DNA sequences may be inserted into the SV40 portion of pSV2 at a unique HindIII site (or between HindIII and BgIII sites) about 70 base pairs from the SV40 origin/early promoter. We have inserted several eukaryotic cDNA and prokaryotic coding segments into pSV2 to generate "secondary" recombinants (pSV2-X). After propagation in E. coli these may be transferred into eukaryotic cells, where the SV40 early region provides promoter, splicing sequences, and termination/polyadenylation site(s) for the generation of insert-specific mRNA. This system should permit stable expression of essentially any insert coding sequence in monkey, human, and mouse cells - i.e. cells in which the SV40 early promoter is constitutively transcribed.

683 SV_{40} VIRAL PROTEINS FACILITATE TRANSCRIPTION OF NUCLEOPROTEIN CORE ISOLATED FROM PURIFIED VIRIONS, John N. Brady, Christian Lavialle, and Norman P. Salzman, NIH, Bethesda, Maryland 20205

The SV_{40} nucleoprotein core released when virions are dissociated in vitro with EGTA-DTT has a sedimentation value of 110-115S and contains viral proteins VP_1 and VP_2 in addition to the proteins found associated with the viral minichromosome, i.e., VP_3 and histones H_2A, H_2B, H_3 and H_4. Under optimal conditions 95-100% of the SV_{40} nucleoprotein cores are able to form transcriptional complexes in the presence of saturating amounts of E. coli RNA polymerase. When sucrose gradients used to purify the virion core are assayed for transcriptional activity, all of the template activity co-sediments with the virion core. If polymerase-core complexes are formed in vitro and then RNA synthesis is allowed to proceed for increasing lengths of time, the ^{3}H-UMP labeled RNA at first co-sediments with 110S virion core; as the length of transcription time increases, a progressive increase in the sedimentation of the SV_{40} cores occur, due to the growth of RNA chains. RNA synthesized from SV_{40} cores sediment as a fairly homogeneous species with a sedimentation value of 16-18S. CsCl density gradient analysis indicates that no loss of protein occurs during the process of transcription. The rate of incorporation of ribonucleoside triphosphates into acid insoluble RNA with SV_{40} cores as the template is 70-95% of that obtained with naked supercoiled SV_{40} DNA. SV_{40} minichromosomes, under identical transcription assay conditions, have an incorporation rate which is 20% of that obtained with naked SV_{40} DNA. These results provide the first suggestive evidence that the "late" viral proteins of SV_{40} may play a role in the regulation of expression of the SV_{40} genome.

684 CHARACTERIZATION OF AN SV40-ASSOCIATED SMALL RNA WHICH CAN ANNEAL TO THE VIRAL EARLY mRNAs. James C. Alwine and George Khoury, National Cancer Institute, N.I.H, Bethesda, Maryland 20205.

We have characterized a small RNA (approximately 70 nucleotides) which is induced late in SV40 lytic infection. This SV40-associated small RNA (SAS-RNA) is specific in size and sequence and is not selected by oligo(dT)-cellulose. The SAS-RNA is complementary to the viral early mRNAs (and the late DNA strand) at a point 260 nucleotides from the 3'-end of the early mRNAs (0.21 map units) and is homologous to at least 50 nucleotides in this region. This area of the genome is interesting because it marks the start of a second open translational reading frame at the carboxy terminal end of T-antigen. It is also the region deleted by ts1499, a deletion mutant which is temperature sensitive for growth but cold sensitive for transformation (Pintel et al, 1979, CSHSQB 44, in press). The function of the SAS-RNA in the viral cycle and its source (host or viral) are unknown at this point; however its temporal expression, unique sequence and its interesting region of homology on the early mRNAs (or the viral genome) suggest a potential role in SV40 gene expression.

685 SV40 LATE LEADER SEQUENCES: STRUCTURE AND FUNCTION, M. Piatak*, M. Olive**, P. Ghosh*, K. N. Subramanian**, P. Lebowitz* and S. M. Weissman*, *Yale Univ. Sch. of Med., New Haven, Ct. 06510 and **Univ. of Ill. Med. Ctr., Chicago, Ill. 60680.

Utilizing the transcription of SV40 as a model system to study the formation and function of leader sequences in mammalian mRNA, we have examined the structure of late viral mRNAs produced in cells infected with each of 3 viable deletion mutants of SV40. These mutants, dl 1659, dl 1626 and dl 1613, lack non-overlapping segments of the DNA encoding the major leaders of late SV40 mRNA. Messenger RNA sequences were analyzed utilizing the reverse transcriptase catalyzed extension of short SV40 DNA fragments hybridized at specific points in the RNA sequence. Among the results are the following: The mutants direct the of synthesis 16S and 19S mRNAs, each type containing splices found in wild-type mRNA. The distribution of the 5'-termini of the mutant mRNAs, however, differs significantly from that of wild-type mRNAs. The abundant forms of mutant mRNAs have 5'-termini which are encoded by DNA sequences lying upstream from DNA encoding the major 5'-termini of wild-type mRNA. A relationship between the selection of 5'-termini and the lengths of the late leaders is suggested. As compared to the abundance of the various forms of wild-type 19S mRNAs, we have found striking differences in the ratio of mutant 19S mRNAs which contain one of the three wild-type splices to those which lack a splice upstream from the body of the message. Cells infected by the mutants dl 1659 and dl 1626 produce an unusually high proportion of the "unspliced" forms of 19S RNA. The possibility that these "unspliced" forms of 19S RNA contain some internal or 3'-terminal splice, not previously detected in wild-type mRNA, is under study.

686 MAPPING OF THE DNA REGION OF POLYOMA STRAIN 3049 RESPONSIBLE FOR THE OVERPRODUCTION OF VIRION-ASSOCIATED PROTEINS. Elaine Kinney-Thomas, J. Donald Hare, University of Rochester, School of Medicine, Rochester New York, 14642

Polyoma strain 3049 produces a two-fold excess of polyadenylated RNA after the onset of DNA replication. The resulting three to five-fold excess of virion proteins accumulates, causing both the cytoplasm and nucleus of infected cells to stain brightly in an indirect fluorescent antibody(FA) assay for viral capsid proteins. The variant phenotype was termed CyC^+. Large plaque Dulbecco(Lp-D), wild type for protein production, causes infected cells to stain only in the nucleus. DNA from the two strains yielded very different HaeIII patterns.The pattern produced by Lp-D was identical to that of strain A2. The HaeIII fragments of strain 3049 were mapped by a combination of techniques, including exonuclease III mapping.

Recombinants were constructed in vitro by joining the DNAs of strains Lp-D and 3049 at the BglI and BamHI sites, at the EcoRI and BamHI sites, and at the two HindIII sites. Unique HaeIII fragments of strain 3049 served as markers to identify those regions of the recombinant genomes composed of 3049 DNA. Analysis of the recombinants by FA defined two possible regions of 3049 DNA responsible for the CyC^+ phenotype. The DNA between coordinates 45.0 and 58.6 in the late region and between 1.0 and 1.4 in the early region of the 3049 genome were common to all CyC^+ clones. The late region(45.0-58.6) contains the 5' splice point of the messenger RNA for viral protein(VP-1)(Kamen, et al, 1978, Virol. 89:461), the entire coding sequence of VP-3 and part of that for VP-2. We interpret these and other data as being consistent with the hypothesis that variant 3049 has an altered splicing mechanism for late messenger RNA.

687 THE FATE OF NEWLY SYNTHESIZED PAPOVAVIRUS DNA, Ann Roman and Hwa-Tang Wang, Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana 46223.

While knowledge of the steps involved in synthesis of papovavirus DNA is quite sophisticated, relatively little is known of the factors determining whether newly synthesized DNA is used as a template for further DNA synthesis (re-enters replication) or whether it becomes encapsidated. We have shown previously that the rate and extent of re-entry of pulse-labeled progeny polyoma DNA is greatest early in the infectious cycle. However, regardless of the time post-infection examined, progeny molecules appear to be removed from the replicating pool approximately three hours after their initial synthesis. Pulse-labeled SV40 DNA re-enters replication over the same time period and then similarly ceases to re-enter. To determine whether encapsidation of pulse-labeled DNA is the direct cause of cessation of re-entry we are examining the kinetics of encapsidation. Preliminary data suggest that as the fraction of pulse-labeled SV40 DNA in previrions and virions increases the fraction of pulse-labeled DNA which re-enters replication decreases. Early in the infectious cycle a smaller percentage of pulse-labeled DNA becomes encapsidated during a six hour chase than late in the cycle. In addition, pulse-labeled DNA is more rapidly encapsidated late in infection. The data suggest that the factor(s) removing viral DNA from the replicating pool (causing cessation of re-entry) is involved in virus maturation.

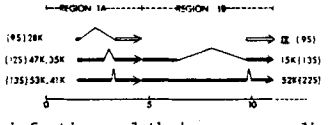
688 AMPLIFICATION OF DEFECTIVE PAPOVAVIRUS DNA's DURING SERIAL LOW MULTIPLICITY PASSAGE IN HUMAN CELL, Frank J. O'Neill and Dana Carroll, Dept. Cellular, Viral and Molecular Biology, Dept. of Pathology, University of Utah and V.A. Medical Center, Salt Lake City, Utah 84148

Previous studies have shown that defective interfering particles of SV40 were generated and amplified when virus was propagated in the human glioblastoma cell line A172, at low multiplicities of infection. The present work shows this is a fairly widespread phenomenon in other human cells with both SV40 and BKV. The human cell lines and cell strains utilized for SV40 and BKV virus growth were fetal brain (HFB), three neuroblastoma's, embryonic kidney (HEK), primary amnion (HAMN), fibroblasts and a variety of melanoma (A101D and A375) and rhabdomyosarcomas. Defectives of SV40 and BKV, appearing as faster moving DNA bands on agarose gels, were present after 3-8 low multiplicity passages in HFB, the three neuroblastomas, a rhabdomyosarcoma cell line and the HEK and HAMN cell strains. Only in the human fibroblasts and the melanoma line A101D were defectives not generated during low multiplicity passage. However, when virus was passed undiluted, defectives did appear. Somewhat similar results were obtained following the growth of polyoma virus in different mouse cell cultures. These results show that high multiplicity passage is not necessary for the amplification of papovavirus defectives. In most cell lines and strains analyzed, serial undiluted passage of SV40 or BKV ultimately resulted in the generation of persistently infected cells including SV40 passed in the green monkey cell line TC-7. These carrier cultures released infectious virus contained both standard and defective free viral DNA's, showed T antigen in 20-100% of the cells but failed to exhibit the transformed phenotype unless cultured for prolonged periods.

689 METHYLATION OF INTEGRATED ADENOVIRUS DNA SEQUENCES AND THEIR EXPRESSION, Walter Doerfler, Diane Sutter, Lily Vardimon, Rainer Neumann and Ingrid Kuhlmann, Institute of Genetics, University of Cologne, Cologne, Germany

Studies on the patterns of integration of adenovirus type 12 (Ad12) DNA have revealed that the integrated viral DNA sequences in Ad12-transformed hamster and in Ad12-induced rat brain tumor cells are extensively methylated (Sutter and Doerfler, 1979). Similar observations have been made for the DNA of adenovirus type 2 (Ad2) in Ad2-transformed hamster cells. In Ad12-transformed hamster cells and in Ad12-induced rat brain tumor cells, an inverse correlation exists between the level of methylation of certain segments of the viral genome and the extent to which these segments are expressed as messenger RNA. Regions of the integrated Ad12 genome, which are silent are completely methylated, regions which are transcribed into mRNA are much less extensively methylated. The DNA in virions of Ad2 or Ad12 does not seem to be methylated (Günthert et al., 1976). When human cells are productively infected with Ad2 or when hamster cells are abortively infected with Ad12, the parental viral DNA purified from isolated nuclei of such infected cells is not strongly methylated, except perhaps at very early times postinfection. Investigations continue to determine in which way methylation of DNA could influence the expression of eukaryotic genes. (Supported by the DFG through SFB74).

690 ADENOVIRUS REGION 1 GENE PRODUCTS. D.N. Halbert, D.J. Spector, and H.J. Raskas, Washington University School of Medicine, Department of Pathology, St. Louis, MO 63110



Early region 1 of adenovirus 2 (0-11%) is necessary and sufficient for transformation of mammalian cells and is involved in regulating productive infection. We have reported the assignment of in vitro translated proteins to individually purified mRNAs within region 1 (Halbert et al., J. Virol. 31: 621, 1979). A composite map of region 1 RNAs synthesized in productive infection and their corresponding protein products is shown in the Figure. Filled arrows represent mRNAs and proteins synthesized early in the infectious cycle; all species are made at late times. The sequence relationships between the region 1 polypeptides translated in vitro has been determined by 2-dimensional peptide mapping. All 5 polypeptides from region 1A are highly related and correspond to proteins isolated from infected cell extracts. The 52K and 15K proteins from region 1B share no methionine peptides. The 12.5K product from region 1B is identical to virion polypeptide IX and shares no methionine peptides with 52K or 15K.

We have examined further the relationship between the 4 polypeptides encoded by the 2 early mRNAs from region 1A. These RNAs have been translated in vitro in the presence of the arginine analog canavanine. Preliminary experiments have revealed higher molecular weight polypeptide products, suggesting that formation of these peptides may include a proteolytic processing event.

691 THE ARRANGEMENT OF THE MESSENGER RNAS AND THEIR PROTEIN CODING SEQUENCES IN THE LATE MAJOR TRANSCRIPTION UNIT OF ADENOVIRUS 2, Jacqueline S. Miller, Robert P. Ricciardi, Bryan E. Roberts, Bruce M. Paterson, and Michael B. Mathews. Harvard Medical School, Boston, Mass.; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; National Cancer Institute, Bethesda, Md.

The mRNAs synthesized late in adenovirus 2 infection have been characterized utilizing three compelmentary methods of analysis. In each case the identity of the mRNA was defined functionally by the polypeptide it synthesized in a reticulocyte cell-free system. These RNAs were characterized by 1)electrophoretic fractionation in agarose gels containing methyl mercury hydroxide to estimate the sizes of the funtional RNAs; 2) selection by annealing to viral DNA fragments immobilized on nitrocellulose to define the genomic origin of their constituent sequences; 3)hybrid arrested translation using viral DNA fragments to locate their protein coding sequence. The data allow the positioning of both the coding and non-coding regions of all the late mRNAs with respect to the DNA, demonstrate that the mRNA sequences overlap within families in a staggered fashion, and reveal the existence of two now late proteins of 52,000 and 55,000 daltons. It has also been demonstrated that certain species of RNAs migrate as discrete species upon electrophoresis in agarose gels containing methyl mercury hydroxide whereas other species exhibit a broad distribution of molecular weights. The significance of this will be discussed.

692 'LEFT END' PROTEINS OF ADENOVIRUSES, M.B. Mathews, H. Esche, J. Smart, B. Stillman, M. Harter, and J. Lewis, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

We have used techniques of cell-free protein synthesis and peptide analysis to analyze the proteins encoded by the transforming region (IA and IB) of adenovirus-2 and -5, and by other early genes located in the left third of the genome. Experiments with virus mutants and with metabolic inhibitors permit identification of some of the functions of these proteins and the regulatory interrelationships involved in the production of their mRNAs. The results to be presented include: (i) the discovery of several 'new' proteins; (ii) the correspondence between the region IA messengers and polypeptides; and (iii) the observation that the major 'late' promoter site is operational during phases of the infection in which it was previously considered quiescent.

693 SELECTIVE GENE EXPRESSION OF ADENOVIRUS DNA MICROINJECTED INTO XENOPUS OOCYTES. Fred A.M. Asselbergs, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724.

Of the ca. 30 proteins which adenovirus encodes, a specific subset, mainly virion components, is synthesized exclusively late in the infection cycle after the onset of viral DNA replication, whereas another subset is already produced early in infection. In Xenopus oocytes microinjected with adenovirus mRNA both early and late proteins were correctly made including some of the post-translational modifications (phosphorylation of the 72 kdalton DNA-binding protein). In contrast, when adenovirus 2 DNA was injected into the (nucleus of) oocytes and oocyte proteins were assayed with specific antisera, only synthesis of the DNA-binding protein (an early protein), but no synthesis of hexon and fiber (two late proteins) could be detected. This observation suggests that oocytes are deficient in the synthesis of functional hexon and fiber mRNA, both of which are generated by splicing from a large precursor RNA initiated at the major late promotor at 16.5 map units. At present the cause of this deficiency is not known, but oocytes are probably not deficient in splicing, because injection of an adenovirus 5 DNA-fragment (HpaI-E, 0-4.5 map units) resulted in the synthesis of a number of proteins with apparent molecular weights of 72,65,24 and 14 kdalton, which are thought to be translation products of differentially spliced mRNAs from the early region 1A. Possibly the lack of expression of late genes in oocytes is due to absence of viral DNA replication in these cells.

694 THE INVOLVEMENT OF ADENOVIRUS EARLY REGIONS IN THE AAV HELPER FUNCTION. B. Carter, C. Laughlin, M. Myers and F. Jay, NIAMDD, National Institutes of Health, Bethesda, Md.

Adeno-associated virus (AAV) grows efficiently only in cells which are infected with adenovirus (Ad). We are analysing mutant adenoviruses to determine which genes may be required for the AAV helper function. Two mutants ts125 and ts107, having temperature-sensitive lesions in the E72 DNA binding protein coded by the Ad early region 2, are deficient for AAV growth at the non_permissive temperature (40°C). In human (KB) cells at 40°C, using either mutant as helper, the accumulation of AAV particles as well as AAV capsid proteins was decreased about two orders of magnitude. In contrast, AAV DNA synthesis was decreased only several fold. Cytoplasmic poly(A)-containing AAV RNA comprises a set of over-lapping spliced RNAs having different 5 start points. With the ts125 mutant as the helper at 40°C there was greatly decreased accumulation of some but not all of these RNAs. Thus, the Ad E72 protein appears to play a positive role in transcription or post-transcriptional processing of some AAV RNAs. This suggests a new function of E72, in addition to those already known. This new function of E72 may be related to its role in the monkey cell host-restriction for adenoviruses. These results further suggest a strong analogy in the pleiotropic functions of the Ad E72 protein and SV40 T-antigen.

Other studies, using the Ad5 ts149 mutant indicate that the Ad5 early region 5 function is not required for the AAV helper function. Additional work using Ad5 deletion mutants (C. Laughlin, N. Jones, T. Shenk, B. Carter, unpublished) indicates that both Ad5 early regions 1a and 1b are required for AAV growth. In contrast to region 2, region 1b is apparently needed for AAV DNA replication.

695 SPONTANEOUS MUTANTS OF ADENOVIRUS-SV40 HYBRID Ad2^{+}ND3 THAT GROW EFFICIENTLY ON AGMK CELLS, Carl W. Anderson, Biology Department, Brookhaven National Laboratory.

Adenovirus 2 fails to replicate efficiently on African green monkey kidney cells due to a block in late virus gene expression, but virus replication can be substantially enhanced by co-infection with SV40. A series of spontaneous mutants of hybrids Ad2^{+}ND3 and Ad2^{+}ND5 have been isolated that grow as efficiently on AGMK cells as on human cells. (Ad2^{+}ND3 and Ad2^{+}ND5 contain SV40 genetic information but do not replicate efficiently on AGMK cells). Three Ad2^{+}ND3 mutants have been partially characterized. Each grows and plaques about as efficiently on AGMK cells as they do on human cells. Each synthesizes late virus proteins, and in particular fiber protein, in infected AGMK cells at levels comparable to human cell infection, but no other change in the pattern of proteins expressed has been noted. Each mutant has a restriction-enzyme digestion pattern identical to the Ad2^{+}ND3 parent for all enzymes tested. Preliminary mapping of one mutant by marker rescue indicates that the mutant is contained in the Eco R1-B restriction fragment. This result suggests that these spontaneous mutants are similar to Ad2^{+}hr400 isolated by D. Klessig (Cell 17[1979]957). Experiments are in progress to further characterize these mutants and to characterize the DNA-binding protein encoded by the region in which these mutants map.

RNA Tumor Virus Genetics

696 ANALYSIS OF INTERMEDIATES IN THE SYNTHESIS OF AVIAN SARCOMA VIRUS DNA, Ronald I. Swanstrom, William DeLorbe, J. Michael Bishop and Harold E Varmus, University of California, San Francisco, California 94143.

Infection of cells with avian sarcoma virus (a retrovirus of chickens) results in the appearance of duplex viral DNA. Linear viral DNA present early after infection contains a non-permuted copy of the RNA template but is longer than the RNA due to a 330 base-pair direct terminal repeat Models describing the genesis of the 330 base-pair repeat have suggested that more than one template is used to synthesize the first strand of DNA. Using both in vitro and in vivo systems, we have examined the structure of two intermediates in DNA synthesis. Synthesis of the first strand of DNA utilizes several templates requiring two transfers of the growing DNA chain. These transfers probably occur through the use of two short direct terminal repeats (each about 20 bases in length). One repeat is present in the viral RNA; its existence and role in DNA synthesis have been documented experimentally. We and others hypothesize that the second repeat appears as complementary sequences at the ends of a replicative intermediate; the evidence for this intermediate is at present circumstantial. These interstrand transfers account for the synthesis of a DNA molecule that is both non-permuted with respect to the RNA template and contains the 330 base-pair direct terminal repeat. The duplex linear viral DNA in turn serves as precursor to covalently closed, circular DNA. We amplified circular viral DNA by molecular cloning and used this material to explore the mechanism by which circular molecules arise. Analysis of nucleotide sequences in the viral DNA indicates a sequence arrangement that bears a striking resemblance to bacterial transposition elements.

697 IDENTIFICATION OF ECOTROPIC VIRAL DNA SEQUENCES IN MOUSE DNA, Hardy W. Chan, Janet Moore, Wallace P. Rowe and Malcolm A. Martin, NIAID, NIH, Bethesda, MD 20014

A specific probe for ecotropic murine leukemia virus DNA sequences was constructed by cloning a segment of AKR viral DNA (previously cloned in lambda charon 4A) using pBR322 plasmid DNA as vector. The cloned DNA contains a 400 base pair segment derived from the env region of the AKR ecotropic virus. Following nick-translation, this probe appears to be specific for ecotropic viral DNA sequences since it fails to react with NFS mouse DNA. We have employed this probe to detect the presence or absence of retraviral DNA sequences in several inbred strains of mice as well as AKR congenic mice using the Southern blotting procedure. These results as well as the construction of probes specific for different regions of endogenous murine leukemia viruses will be discussed.

698 RESTRICTION MAPS OF MURINE MAMMARY TUMOR PROVIRAL SEQUENCES CORRELATE WITH GENETIC SEGREGATION OF SUSCEPTIBILITY TO MAMMARY TUMORIGENESIS, W. Drohan and J. Young and J. Schlom, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

We have investigated the mouse mammary tumor virus (MMTV) specific proviral DNA content of the C57BL and GR strains of mice by restriction endonuclease digestion of cellular DNA and Southern transfer of DNA fragments to nitrocellulose filter paper. GR mice develop 100% mammary tumors at an early age whereas C57BL mice have less than a one percent incidence. The MMTV containing DNA fragments were identified by hybridization to MMTV [^{32}P] complementary DNA. Digestion of cellular DNA with the restriction endonuclease Pst1, which generates multiple internal MMTV specific DNA fragments, has identified viral DNA fragments at 2.5 and 0.6 megadaltons in the GR DNA, which are absent in the C57BL DNA. The GR and C57BL mice share Pst1 restriction fragments at 3.3, 1.1, 0.9 and 0.5 megadaltons in common. The C57BL mouse has a Pst1 restriction fragment at 3.1 megadaltons which is absent in DNA from GR mice. Genetic analyses of GR and C57BL crosses has determined that the Pst1 fragments specific for the GR MMTV are inherited as a single Mendelian unit in [C57BL x (C57BL x GR)F_1] backcross mice, i.e., 50 percent of the livers examined contain Pst1 fragments at 2.5 and 0.6 megadaltons. Furthermore, we have been able to correlate the presence of these MMTV(GR) specific Pst1 fragments with the occurrence of early mammary tumors in the [C57BL x (C57BL x GR)F_1] backcross mice. These studies thus demonstrate the utility of restriction mapping in the diagnosis of a naturally occurring neoplasm.

699 DIFFERENTIAL METHYLATION OF ENDOGENOUS AND ACQUIRED MOUSE MAMMARY TUMOR VIRUS-SPECIFIC PROVIRAL DNA, J. Craig Cohen, Tulane University Medical Center, New Orleans, LA, 70112

Using the restriction endonucleases Msp I and Hpa II, isochisomers which distinquish methylated from non-methylated sites (CCGG), and the DNA blotting techinque, DNAs from uninfected and mouse mammary tumor virus (MMTV) infected tissues of various inbred mouse strains were analyzed to determine the 5-methylcytosine content of proviral DNA. Genetically-transmitted, endogenous, virus-specific sequences were found to be extensively modified regardless of the inbred mouse strain or MMTV genotype. When DNA from tissues infected with the milk-borne, acquired MMTV was examined, acquired viral sequences were hypomethylated while the endogenous proviruses remain modified. Characterization of DNA from tumors of numerous infected strains revealed similar results. In addition to the Msp I-Hpa II site, the enzyme Hha I, which is inhibited by methylation at GCGC sites, was also used to extend and confirm these observations. This phenomenon was found to be independent of viral sequence and physiological state of the cell, since it was observed in tissues in which the endogenous and acquired MMTV proviruses are identical and since it occurred in MMTV infected, normal lactating mammary gland as well as in all mammary carcinomas tested.

700 DNA SEQUENCE OF INDEPENDENT MMTV INTEGRATION EVENTS REVEALS LACK OF SITE-SPECIFIC INTEGRATION, Gordon L. Hager and Lawrence A. Donehower, Tumor Virus Genetics Lab, N.C.I., N.I.H., Bethesda, Md. 20014.

An important aspect of the regulation of retrovirus expression concerns the interaction, if any, between host cell and viral sequences. It has been shown by restriction enzyme analysis in a number of retroviral systems that exogenously acquired genomes are integrated at a specific site with respect to the viral genome, but at a large number of sites in cellular DNA. Thus the mechanism of integration either is nonspecific vis-a-vis primary sequence, or many copies exist of a recognition sequence too small to be detected by restriction analysis. We have applied DNA sequence analysis to resolve this question in the MMTV system. Molecular clones of newly integrated MMTV genomes in a cell line derived from a C3H mammary carcinoma were isolated by recombinant DNA techniques. Fragments containing the virus-cell "joints" at the 3' end of the integrated genomes were prepared and subjected to sequence analysis. These sequences were compared with the 3' end of unintegrated MMTV DNA as inferred from the MMTV "strong-stop" sequence determined by Lovinger and Schochetman (personal communication). Although 3' ends of integrated MMTV genomes contain greater than 98% homology, and agree with the MMTV "strong-stop" sequence, no homology whatsoever is detected between cell-specific sequences lying outside the joint defined by the first MMTV-specific nucleotide. These results imply that the mechanism of integration does not involve sequence recognition. Furthermore, these observations do not favor models invoking the promotion and regulation of retroviral expression from specific cellular sequences.

701 INTEGRATED AND PROVIRAL DNA SEQUENCES SPECIFIC FOR A WILD RAT TUMOR VIRUS, WR-RaLV, AND FOR RAT HEPATOMA HELPER VIRUS, RHHV, IN VARIOUS LABORATORY AND WILD RAT TUMOR CELLS. Stringner S. Yang[1], J. Taub[1], L-S. Yeh[1], S. Rasheed[2] and M. Gardner[2], Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD 20205[1] and University of Southern California, Los Angeles, CA 90033[2].

We have identified and isolated the viral specific DNA sequences for a wild rat tumor virus, WR-RaLV, and for rat hepatoma helper virus, RHHV, carried in the integrated nuclear DNA fragments as well as the unintegrated proviral DNA prepared from the Hirt supernatant by using a multidisciplinary approach combining reverse phase V column chromatography, restriction endonuclease analysis, Southern blot transfer and nucleic acid hybridization. They measure 8.0 kbp and 8.6 kbp respectively and are both in linear form. Both the proviral DNA sequences agree closely with the cellular integrated DNA sequences specific for each virus; i.e. 8.4 kbp for WR-RaLV in wild rat tumor cell (WRT) DNA and 8.4-8.8 kbp for RHHV in the DNA of the KiMSV(RHHV) transformed tumor cells. WR-RaLV integration site seems to be unique and singular in the WRT cell DNA. On the other hand, multiple integration sites are observed for KiMSV(RHHV) specific sequences in the DNA of several KiMSV(RHHV) transformed tumor cell lines. In these latter tumor cell lines the integrated DNA sequences specific for KiMSV were also identified as a separate 6.4 kbp band. Secondary digestions by other restriction endonucleases of the 8.4 kbp band specific for WR-RaLV or RHHV, such as Hpa II, Bam Hl and others resulted in several small DNA fragments. A preliminary map for the WR-RaLV and RHHV genomic DNA sequences will be presented.

702 DISPOSITION OF ECOTROPIC PROVIRUSES IN AKR MICE, Winship Herr, Dennis Schwartz, and Walter Gilbert, Harvard University, Cambridge MA 02138

AKV is an ecotropic virus carried endogenously by AKR mice. We are interested in the disposition of this virus in the AKR genome and have addressed the problem by Southern blot analysis of restricted genomic DNA using a variety of AKV cDNA probes specific for the env gene (GP70) and surrounding regions. These probes are restriction fragments which have been identified by comparing the restriction map of the 3' region of AKV with that of an MCF virus containing an altered env gene. Such fragments yield simple hybridization patterns which readily characterize different AKR/J genomes with respect to the number, location and integrity of this endogenous virus.

Embryonic, adult and leukemic tissues of AKR/J mice have been analyzed and compared with the following results: AKR/J mice have different germ line patterns with varying numbers of proviruses present. Leukemic thymuses display additional viral sequences in which the ecotropic viral integrity is affected.

703 GENETIC MAPPING OF THE ECOTROPIC MURINE LEUKEMIA VIRUS-INDUCING LOCUS (Akv-2) OF AKR MICE, Christine A. Kozak and Wallace P. Rowe, National Institutes of Health, Bethesda, MD 20205

The techniques of somatic cell hybridization and mendelian breeding studies were combined to map the ecotropic virus-inducing locus of AKR mice, Akv-2, to the centromeric end of mouse chromosome 16. Somatic cell hybrids were made between cells of the Chinese hamster line, E36, and spleen cells of 10-day old mice congenic for Akv-2. Thirty-one primary hybrid clones and 36 secondary clones were induced for ecotropic virus by 5-iododeoxyuridine. A comparison of virus induction and expression of isozyme markers on 14 mouse chromosomes showed concordance with only one marker, superoxide dismutase (Sod-1) on chromosome 16. Genetic crosses between Akv-2 congenic mice and mice carrying the mahoganoid coat color locus (md) on chromosome 16 showed close linkage between these markers. Additional crosses have identified ecotropic virus-inducing loci on 5 additional chromosomes in other mouse strains. The assignment of Akv-2 further emphasizes that endogenous ecotropic retroviruses are inserted at multiple sites in mouse chromosomes.

704 STUDY OF ENDOGENOUS MULV-RELATED SEQUENCES IN MOUSE CELLS, Hung Fan, David S. Goldberg & Lee T. Bacheler, The Salk Institute, San Diego, Ca. 92138

Mouse cells carry DNA sequences related to murine C-type viruses in multiple copies. If mouse cell DNA is cleaved with Eco RI restriction endonuclease (which cleaves most known MuLV DNA's none or only a few times), a large number of MuLV-related restriction enzyme fragments are observed, indicating that the endogenous MuLV-related sequences consist of many copies in a large number of chromosomal sites. We have studied mouse cell DNA using Kpn I restriction endonuclease; Kpn I cleaves Moloney MuLV DNA close to both termini of unintegrated viral DNA as well as several times internally. Digestion of uninfected mouse cell DNA with Kpn I resulted in a greatly simplified pattern of MuLV-related fragments, suggesting that the endogenous MuLV-related sequences consist of a small number of quite related retroviruses integrated into many different chromosomal sites. Cleavage with two other enzymes which also cleave M-MuLV DNA very close to the viral DNA termini, Sac I and Pvu II, also produced very simple patterns of M-MuLV-related fragments. Sequential digestion with Kpn I and RI indicated that three major classes of MuLV-related sequence organizations exist. Comparison of several inbred mouse strains as well as a feral isolate suggest that most of the MuLV-related sequences were acquired into the germline of mice before the derivation of modern inbred strains. Several different lambdaphage recombinant DNA clones containing the 5' portions of endogenous retroviruses as well as their adjacent sequences have been isolated.

705 CLONING OF INTEGRATED MOLONEY MURINE LEUKEMIA VIRUS AND RELATED DNA SEQUENCES FROM INFECTED MOUSE CELLS, Lee Bacheler and Hung Fan, Salk Institute, San Diego, Ca. 92138.

Eco R_I DNA fragments from mouse cells infected with M-MuLV have been cloned in the Charon 4A lambda phage cloning vector in order to derive DNA clones containing integrated Moloney murine leukemia virus (M-MuLV) DNA. Those clones which hybridize with an M-MuLV cDNA probe were selected. In addition to integrated M-MuLV DNA genomes, clones of a family of endogenous sequences related to M-MuLV which are contained in 30-40 different Eco R_I fragments of uninfected mouse cell DNA will be recognized by this screening procedure. To enrich Eco R_I cut cellular DNA for integrated M-MuLV sequences, DNA has been size fractionated by preparative agarose gel electrophoresis and screened, prior to cloning, for the presence of M-MuLV DNA sequences by cleavage with restriction enzymes which release uniquely characteristic fragments from M-MuLV DNA, and in some cases, by assays of biological activity (XC plaque production) following transfection of DNA fractions. A large number (more than 50) of M-MuLV-related clones have been isolated. At least four different clones contain an M-MuLV-related 1.8×10^6 dalton Kpn I fragment which is also found in M-MuLV DNA. These clones apparently represent different MuLV-related sequences, possibly the 5' half of a common virus-like sequence organization. In addition, a number of unstable, M-MuLV-related clones have been isolated, which appear to delete portions of the inserted sequences.

706 STRUCTURAL AND GENETIC RELATIONSHIPS BETWEEN AN ENDOGENOUS RETROVIRUS (M432) OF MUS CERVICOLOR AND INTRACISTERNAL A-PARTICLES OF MUS MUSCULUS. R. Callahan, E.L. Kuff, K.K. Lueders and E. Birkenmeier. National Cancer Institute, Bethesda, MD 20205.

A novel class of endogenous retrovirus has been isolated from the Asian murine species Mus cervicolor; the prototype virus, M432, has been shown by immunological and biochemical criteria to be unrelated to type-B and type-C murine retroviruses (Callahan et al. Virology 80:401-416, 1977). Recently the genome of intracisternal type-A particles (IAP), retrovirus-like entities seen in many tumors of Mus musculus, was found to share partial sequence homology with the M432 viral RNA (Kuff et al. J. Virol. 28:66-74, 1978). We have now shown that antisera directed against the 73,000 dalton major structural protein of IAP specifically precipitates the main internal M432 viral protein, p24. In reciprocal experiments, antisera prepared against both disrupted whole M432 virus or purified p24 precipitate the IAP p73. We have used this immunological cross-reactivity to develop an interspecies competition radioimmunoassay. Other classes of mammalian endogenous retroviruses do not compete in this assay. Other potential relationships between IAP proteins and those of the M432 virus or their precursors are under study.

Nucleic acid sequences related to the M432 and IAP genomes are found in multiple copies in cellular DNA from members of the genus Mus. The organization of these sequences in the DNA of individuals of a feral population of M. cervicolor and various inbred strains of M. musculus has been studied using restriction endonucleases and the Southern blotting technique. The results indicate that the arrangement of viral sequences is well-conserved within members of each species, whereas major differences are seen when different species are compared.

707 PARTIAL TYPE-C PROVIRUS IN AN INFECTED GIBBON APE, Marvin S. Reitz, Flossie Wong-Staal, Maribeth Voltin and Robert C. Gallo, NIH, Bethesda, Maryland 20205

DNA from different tissues of a gibbon ape infected with gibbon ape leukemia virus (GaLV) were examined for the presence of GaLV proviral sequences. The gibbon examined (6G-4) was exposed to a leukemic, highly viremic, gibbon and was apparently infected with GaLV, as manifested by the presence of persistent serum antibodies to several viral proteins (Gallagher et al., J. Nat. Cancer Inst. 60:677, 1978; Krakower et al., Int. J. Cancer 22:715, 1978). However, gibbon 6G-4 was not viremic, expressed no detectable viral proteins, and had no apparent hematopoietic abnormalities. Proviral sequences were detectable in DNA from kidney, spleen, and liver of 6G-4, but not from brain, muscle, bone marrow, heart, or other tissues tested. The kinetics of hybridization and the banding pattern on agarose gels of provirus-containing restriction nuclease fragments indicate that the provirus contains a substantial deletion of the viral genome. Use of cDNAs specific to particular regions of the viral genome show that the partial provirus in the liver consists primarily of sequences from the extreme 5' end of the genomic RNA and the 3' 30-40% of the RNA. This partial provirus is present at about one copy per haploid genome. A complete or nearly complete provirus is also present, but only at approximately one copy per thirty haploid genomes. The data thus show the establishment via a natural infection of an integrated partial provirus of a type-C virus. If the gene order of $GaLV_H$ is like that of avian and murine type-C viruses, the detected sequences would correspond primarily to the env gene.

708 INHIBITION OF RNA-DEPENDENT DNA POLYMERASE ACTIVITY OF ONCORNAVIRUSES BY CAFFEINE, A. Srinivasan, E. Premkumar Reddy and P. S. Sarma, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20205

The effect of caffeine on reverse transcriptase (RNA-dependent DNA polymerase) of mammalian and avian oncogenic viruses has been studied. Caffeine inhibited reverse transcriptase activity of Rauscher leukemia virus when endogenous viral RNA and poly(rA)·$(dT)_{12-18}$ were used as templates. Similar results were also obtained with purified reverse transcriptase of avian myeloblastosis virus utilizing 70S and 35S RNA of AMV, poly(rA)·$(dT)_{12-18}$ globin mRNA and activated calf thymus DNA as templates. The inhibitory effect was dependent on the template used and was evident only when it was present during the initiation of polymerization reaction. Increasing the template concentration in the reaction mixture partly reversed the effect of caffeine. These results will be discussed in the light of the mechanism of action of caffeine.

709 NICKING-CLOSING ENZYME ACTIVITY ASSOCIATED WITH ROUS SARCOMA VIRUS:
John H. Weis, Anthony J. Faras, University of Minnesota, Minneapolis, MN 55455

A nicking-closing topoisomerse, swivelase, has been discovered associated with the virions of avian retroviruses. The swivelase activity co-purifies with the viral particles on sucrose gradients and is maximal with non-ionic detergent disruption of the viral envelope. The Rous sarcoma virus associated swivelase has been purified from the virus by ion-exchange and affinity chromotography. Swivelase is distinct from the viral polymerase, reverse transcriptase, since the two activities can be clearly separated by the purification procedure. The apparent molecular weight of the enzyme is 75,000 daltons as assayed by glycerol gradient centrifugation. Swivelase activity is greatest at 0.2M NaCl(KCl), does not require divalent cations, and is ATP independent. The enzyme does facilitate the sequential loss of superturns in a supercoiled DNA substrate, Col El plasmid, thus differentiating its action from that of an endonuclease present within the virus. Both negative and positive supercoiled DNA circles can be relaxed by the enzyme.

710 AVIAN RETROVIRUS POLYMERASE ASSOCIATED ENDONUCLEASE, Duane P. Grandgenett, Ajaykumar C. Vora, Miriam Golomb, William S. Masont, and Tapan K. Misra, St. Louis University Medical Center, Institute for Molecular Virology, St. Louis, MO. 63110, †Institute for Cancer Research, Philadelphia, PA. 19111

Studies were initiated to characterize the endonuclease activities associated with avian retrovirus αβ DNA polymerase and $p32^{pol}$. Partial chymotryptic digestion of purified avian myeloblastosis virus (AMV) αβ DNA polymerase resulted in the activation of a Mg^{2+}-dependent DNA endonuclease activity. Incubation of the polymerase-protease mixture in the presence of supercoiled DNA and Mg^{2+} permitted detection of the cleaved polymerase fragment possessing DNA nicking activity. We have demonstrated that this group of purified polymerase fragments derived by chymotryptic digestion of αβ DNA polymerase is similar to the *in vivo* isolated AMV $p32^{pol}$ in size, sequence, and DNA endonuclease activity. Further studies will be presented describing the strand and site-specificity of the αβ DNA polymerase and $p32^{pol}$ endonuclease on supercoiled DNA molecules. The thermolability of the DNA endonuclease activity associated with purified αβ DNA polymerase from various temperature-sensitive mutants of Rous sarcoma virus will also be discussed.

711 VARIABILITY OF INTERFERON EFFECT ON MURINE LEUKEMIA VIRUS, Christine W. Czarniecki, Amos Panet, Robert M. Friedman, Laboratory of Experimental Pathology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205.

Interferon (IF) affects the production of murine leukemia viruses in chronically infected cells. In some systems, IF treatment inhibited virus release while in others, the virions released were deficient in infectivity. To determine whether the observed differences in IF effect were dependent on the infecting virus or the host cell, a clone of Maloney murine leukemia virus (M-MuLV) was used to establish persistent infections in several mouse cell lines. All of these cell lines were capable of establishing an antiviral state, as shown by resistance to challenge by EMC virus in the standard CPE test after IF treatment. Our data indicated that IF treatment had no effect on the production of M-MuLV in NIH 3T3 cells, as determined by reverse transcriptase activity and infectivity of virus released into the culture fluid. In contrast, IF treatment of two separate clones of Swiss 3T3 cells resulted in significant decreases in the reverse transcriptase activity and infectivity of released virions. Additionally, preliminary data indicated that low levels of non-infectious particles were released from one of these clones after IF treatment. The varied effects of IF on the production of M-MuLV were further characterized by analyzing immunoprecipitated viral proteins in infected cells as well as in released virions. These results show that the extent and type of inhibition of M-MuLV production caused by IF is related to the host cell used for infection.

712 EFFECT OF CELL CYCLE ON THE EXPRESSION OF MURINE LEUKEMIA VIRAL GENES IN CHRONICALLY INFECTED CELLS, Amos Panet,*+ Naomi Guttman,* and Howard Cedar,* Department of Virology*, The Hebrew University, Jerusalem, Israel and Laboratory of Experimental Pathology+, National Institutes of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Md., U.S.A.

We have developed a technique for the isolation and characterization of newly transcribed murine leukemia viral RNA in chronically infected cells. Cellular RNA was pulse labeled with ^{3}H-uridine and virus specific sequences were annealed with an excess of mercurated complementary DNA. Based on the affinity between mercurated cDNA and sulfhydryl-Sepharose, the hybrid was specifically selected by affinity column chromatography. The specificity of this method was dependent on the purity of the cDNA, and it was necessary to remove non-viral sequences from the cDNA in order to isolate virus-specific RNA.

Using this methodology, we have investigated the effect of cell cycle on the transcriptional activity of proviral genes. Cultures of Moloney MuLV infected rat cells arrested in Go phase of the cell cycle released reduced quantities of virus, but continued to synthesize viral RNA. The pools of viral RNA and p30 antigen in the Go arrested cells equalled the pools in actively dividing cells. These results suggested that post-transcriptional events controlled virus production in the Go arrested cells.

713 BIOCHEMICAL EVENTS ASSOCIATED WITH THE REPLICATION OF MURINE LEUKEMIA VIRUS, Samuel Salzberg, Revital Shurtz, Ayala Bank and Mordechai Aboud, Department of Life Sciences, Bar Ilan University, Ramat-Gan, Israel

Early biochemical events associated with the replication of the Moloney strain of murine leukemia virus (MLV) was studied in infected NIH/3T3 cells. Parental-polyadenylated viral RNA molecules are detected in polyribosomes with a great abundance at 3 hours after infection. These molecules are released from polyribosomes with EDTA or if infection occurs in the presence of cycloheximide. Size analysis on the polyribosomal viral RNA indicates that 38S and 23S molecules are evident.

The kinetics of cytoplasmic viral DNA synthesis was followed by thymidine labeling of MLV-infected cells. An increased rate of synthesis is observed up to 3.5 hours after infection followed by a decrease. Infection of interferon-treated cells results in a reduced rate of viral DNA synthesis. Preliminary results show that a cellular factor, probably a protein, is involved in the synthesis of viral DNA. Its amount is increased in infected cells.

714 ANALYSIS OF RETROVIRUS MATURATION AND ASSEMBLY, Paula Traktman and David Baltimore, M.I.T., Cambridge, Mass. 02139

We have investigated the steps involved in murine retrovirus maturation using temperature-sensitive and deletion mutants. By analysis of doubly infected clones, we have been able to study the complementation patterns between various mutant and wild-type viruses. We have obtained genetic evidence for a virally coded, trans-acting factor involved in the proteolytic processing of $pr65^{gag}$. We have also found that protease treatment of intact cells can effect a bypass of the maturation and assembly block in a late temperature-sensitive mutant of MuLV. Finally, we are investigating various polymerase mutants of MuLV, both those with thermolabile enzyme activities and those defective in $pr180^{gag-pol}$ processing.

In some cases, we have obtained wild-type recombinant viruses from doubly infected clones. These recombinants have been mapped with respect to their protein products and their nucleic acid inheritance. The in vivo leukemogenicity of the parental and recombinant viruses has been investigated. Preliminary results indicate that these experiments will aid in mapping the viral functions that determine the target cells and organs of the MuLV-induced leukemias.

715 CHARACTERIZATION OF 35S GENOMIC AND POLYSOMAL RSV RNAs, Peter A. Bromley, Jean-L. Darlix and Pierre-F. Spahr, University of Geneva, 1211-Genève 4

We have previously demonstrated the use of mercurated RSV strong-stop cDNA for the isolation of RSV-specific mRNA from infected cells (Bromley et al., 1979, J. Virol. 31, 86-93). We will present the results of cloning experiments using strong-stop DNA, designed for the large scale isolation of avian tumor virus specific mRNAs from infected and uninfected cells.

We present an analysis of the structure of RSV genomic and RSV-specific 35S polysomal RNA with respect to the 5'-end, up to and including the initiation site for GAG protein synthesis. The methodology employed for sequence analysis and ribosome interaction in vitro with the 5'-end of genomic and 35S polysomal RNA will be reported. The implication of the 5'-end sequence arrangement on the nature of in vitro translation products immunologically related to the GAG protein will also be discussed.

716 IN VITRO PROCESSING OF MOL-MLV 35S RNA, John A. Ptak and P.K.Y. Wong, University of Illinois, Urbana, Illinois 61801

The study to be presented indicates that the Mol-MuLV phosphoprotein p12 (pp12) plays a regulatory role in the in vitro processing of MLV 35S RNA by a RNAse III-like activity. These results demonstrate that pp12 can inhibit the processing of 35S RNA to a 1.7×10^6 dalton poly(A)-containing product and a 1.5×10^6 dalton non-poly(A) product by binding to RNAse III-sensitive double-stranded regions of the viral RNA. The major ribonucleoprotein of MuLV--p10--has no effect on the in vitro processing. The RNAse III-like activity has been isolated from a mouse thymus-bone marrow cell line. The presence of a cap I structure on both 35S RNA and the 1.7×10^6 dalton product and the possible significance of these results in relation to mRNA splicing will be discussed.

717 SUPPRESSION OF MURINE RETROVIRUS POLYPEPTIDE TERMINATION: THE EFFECT OF AMBER SUPPRESSOR tRNA ON THE CELL-FREE TRANSLATION OF R-MuLV, Mo-MuLV, AND Mo-MuSV 124 RNA, E. C. Murphy, Jr., and R. B. Arlinghaus, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

The effect of suppressor tRNAs on the cell-free translation of several leukemia and sarcoma virus RNAs has been examined. Yeast amber suppressor tRNA (amber tRNA) enhanced the synthesis of the Rauscher murine leukemia virus (R-MuLV) and clone 1 Moloney leukemia virus (Mo-MuLV) $Pr200^{gag-pol}$ polypeptide by 10-45-fold while at the same time depressing the synthesis of R-MuLV $Pr65^{gag}$ and Mo-MuLV $Pr63^{gag}$. Amber tRNA also slightly depressed the synthesis of R-MuLV $Pr75^{gag}$ and stimulated the synthesis of Mo-MuLV $Pr70^{gag}$. Yeast ochre suppressor tRNA appeared to be ineffective. Quantitative analyses of the kinetics of viral precursor polypeptide accumulation in the presence of amber tRNA showed that during linear protein synthesis, the relative molar increase in accumulated Mo-MuLV $Pr200^{gag-pol}$ and $Pr70^{gag}$ matched nearly exactly the molar loss of $Pr63^{gag}$. Enhancement of $Pr200^{gag-pol}$ and $Pr70^{gag}$ by amber tRNA persisted in the presence of pactamycin, a drug that blocks the initiation of protein synthesis, thus arguing for the addition of amino acids to the C-terminus of $Pr63^{gag}$ as the mechanism behind the amber tRNA effect. Mo-MuLV 124 30S RNA was translated into four major polypeptides, $Pr63^{gag}$, P42, P38, and P23. In the presence of amber tRNA, a new polypeptide, $Pr67^{gag}$, appeared, while $Pr63^{gag}$ synthesis was decreased. Quantitative estimates indicated that for every mole of $Pr67^{gag}$ that appeared, one mole of $Pr63^{gag}$ was lost.

718 PACKAGING OF AN ABUNDANT HOST CELL mRNA BY ROUS SARCOMA VIRUS, Becky Adkins and Tony Hunter, The Salk Institute, San Diego, CA 92138

The messenger activities of 70S virion RNAs from a variety of avian leukosis-sarcoma viruses have been examined by in vitro translation. A polyadenylated 18S RNA which codes for a polypeptide of 34,000 daltons has been found in all non-defective sarcoma viruses analyzed. It is the only major messenger activity other than the 35S genomic RNA found in a transformation defective deletion mutant. Tryptic peptide analysis of methionine containing peptides has revealed that the 34K proteins synthesized in response to different virion RNAs are identical. RNA from uninfected SPAFAS gs-chf- chick embryo fibroblasts contains an abundant messenger activity for a polypeptide which co-migrates in SDS polyacrylamide gels with the 34,000 dalton protein made in response to virion RNA. The tryptic peptide map of this 34K protein is identical with the virion RNA 34K protein. In addition, the uninfected cell RNA 34K shares 3 methionine containing tryptic peptides with Pr76, the precursor to the virion internal structural proteins. These observations suggest that the 34K messenger activity found in 70S virion RNA is the result of selective packaging of an abundant host cell mRNA. We are investigating the possibility that this RNA represents a transcript of a deleted endogenous viral genome.

719 IN VITRO TRANSLATION OF MOUSE MAMMARY TUMOR VIRUS RNA. Gordon Peters and Clive Dickson Imperial Cancer Research Fund, P.O.Box 123, London, United Kingdom.

The mouse mammary tumour virus (MuMTV) genome is a single-stranded 35S RNA of positive sense, containing the 5' capped structure and 3' poly-A segment characteristic of most eukaryotic messenger RNAs. As with other retroviruses the genome of MuMTV is thought to encode at least three genes required for viral replication. In an attempt to determine whether the genome carries information for proteins not involved in structural and replicative functions but which may be responsible for the oncogenic potential of the virus, we have translated MuMTV genomic RNA in a messenger dependent rabbit reticulocyte lysate. Within mature virions, the genomic 35S RNA occurs as a dimer associated with some cellular tRNAs in a 70S complex. Thermal denaturation of this complex releases the genome length subunits together with a spectrum of smaller RNA species most of which are presumed to be derived by random degradation of the genome RNA. Following selection of poly-adenylated RNAs by poly-U-Sepharose chromatography, the various sized RNAs were separated by sucrose gradient sedimentation and individual fractions translated in the cell free system. Translation of full length 35S RNA resulted in synthesis of the precursor polypeptides for the internal structural proteins of the virus and the presumptive polymerase precursor, placing these genes in the 5'-proximal region of the genome, analogous to other retroviruses. From RNA of about 12-16S in size, a series of small, related products were obtained which are distinct from the previously characterised structural proteins, envelope glycoproteins or DNA polymerase. We are currently examining these products further to determine whether they can be implicated in the transforming function of MuMTV.

720 PROTEIN STRUCTURE AND Fv-1 HOST RESTRICTION, Swadesh Duttagupta and Ruy Soeiro. Albert Einstein College of Medicine, Bronx, New York 10461.

Biological studies of Fv-1 restriction suggest a viral protein target for the host effector molecule. Earlier studies had shown specific changes in p 30 on host range conversion. We have cloned a series of separately derived host range varients of B-tropic Friend LLV which exhibit NB host range. The core proteins of these varients as well as their B-tropic parent have been identified in poly-acrylamide gels by a combination of immunoprecipitation, molecular weight, and phosphorous content as p 30, p 15, p 12, and p 10. Iodination followed by digestion with trypsin and 2-dimensional tryptide analysis of each of these proteins has been carried out. Our data confirm earlier results of Gautsch et al on the changes in p 30. We extend their results to show that while p 10 reveals no differences, p 15 and p 12 appear also to show structural alteration on NB conversion.

721 HETEROGENEITY OF THE MAJOR CORE PROTEIN OF MURINE LEUKEMIA VIRUSES, Stephen Louie, Cancer Research Center, Tufts University School of Medicine, Boston, MA 02111.

Analysis by two-dimensional isoelectric focusing--SDS polyacrylamide gel electrophoresis of MuLV proteins revealed interstrain and intrastrain heterogeneity of the major core protein p30. All viruses analyzed had one major and at least one minor p30 component, with regard to isoelectric point (pI).

Virus	Host Range	Fv-1 Tropism	pI of p30 components	
			Major	Minor
Rauscher	Ecotropic	NB	6.7	6.3, 6.2, 6.0
Moloney	Ecotropic	NB	6.5	6.3
AKR	Ecotropic	N	6.7	6.6, 6.3, 6.2
BALB Virus 1	Ecotropic	N	6.7	6.3
BIO.A	Ecotropic	B	6.3	6.1
CAF_1	Ecotropic	B	6.3	6.1
BALB Virus 2	Xenotropic	?	6.3	6.1
HRS/J	Xenotropic	?	6.3	6.1
HRS/J	Polytropic	N	7.1	6.7, 6.4, 6.3

The p30's of B-tropic and xenotropic viruses have similar pI's, and lack the more basic components present in N-tropic and NB-tropic viruses. Though only a limited number of viruses have been analyzed, the pattern that has emerged supports recent evidence suggesting a relationship between Fv-1 tropism and p30, and the possible recombinational origin of some B-tropic viruses from xenotropic viruses.

Herpes and Pos Virus

722 IDENTIFICATION OF THE HERPES SIMPLEX VIRUS (HSV) GLYCOPROTEINS WITH TYPE-SPECIFIC AND CROSS-REACTING NEUTRALIZING SITES BY THE USE OF MONOCLONAL ANTIBODY Lenore P. Pereira and J. Richard baringer, Veterans Administration Medical Center, San Francisco, CA 94121

Monoclonal antibody with neutralizing activity for HSV-1 and HSV-2 was used to identify the viral glycoproteins which bind neutralizing antibody. Five hybrids which produced neutralizing antibody of the IgG_1 or IgG_{2a} subclass were cloned. In comparative reactions they were found to differ in their neutralizing activity for HSV-1 and HSV-2. Three clones were characterized by antibody which reacted with HSV-1 while two clones produced antibody which neutralized both type 1 and type 2. Fluorescent staining reactions showed that the binding of monoclonal antibody to antigens expressed on the surface of HSV-infected cells correlated with the pattern of neutralization. In immune precipitation reactions with soluble antigen preparations, two glycoproteins were precipited by monoclonal antibody of different site specificities. Glycoprotein C_2 (137,000 MW) specifies neutralizing sites which are specific for type 1. In contrast, glycoptotein D_2 (55,000 MW) specifies neutralizing determinants which are shared by type 1 and type 2. In addition this glycoprotein also appears to specify determinants which bind neutralizing antibody specific for HSV-1.

723 CHARACTERIZATION OF THE IMMEDIATE EARLY mRNA OF HERPES SIMPLEX VIRUS 1. Robert Millette and Susan Talley-Brown, Department of Immunology and Microbiology, Wayne State University School of Medicine, Detroit, Michigan 48201.

Immediate early (IE) mRNAs of herpes simplex virus 1 (HSV-1) that accumulate in the cytoplasm of infected cells in the presence of cycloheximide were identified by hybridization to HSV DNA cellulose and by electrophoresis in denaturing gradient polyacrylamide gels. Four IE mRNA bands were observed, numbered 1 - 4 respectively, having lengths of 5.2, 3.8, 2.7, and 1.8 kilobases(kb). By hybridization and in vitro translation of $poly(A)^+$ and $poly(A)^-$ RNA we find that at least 80% of the IE viral RNA is polyadenylated. Furthermore, fractionation on poly(U) columns indicates that almost all of the IE poly(A) RNA is in the $poly(A)_{150}$ class.

Individual RNAs, isolated by sucrose gradient centrifugation or by elution from gels, were translated in vitro to determine their coding capacity. The results show that viral polypeptides (VP) 145,165, and 123 ($M \times 10^{-3}$) are encoded by RNAs 1,2, and 3 respectively, and VP 86,71, and 55 are encoded by RNA 4.

The size of RNAs shows close coincidence with the UV target size determined for expression of the corresponding genes from irradiated viral DNA. Thus extensive intervening sequences are not involved in the expression of these particular viral genes. Furthermore the UV target sizes for the expression of the IE genes for VP 165,145,123, and 55 indicate that these genes reside in separate transcription units. To establish map coordinates of their DNA coding sequences and possible intervening sequences, the individual mRNA species have been further characterized by Southern blot and R-loop mapping and by electrophoretic analysis of hybridized DNA fragments.

724 CORRELATIVE ANALYSIS OF HERPESVIRUSES BY TWO-DIMENSIONAL ELECTROPHORESIS OF PROTEINS AND RESTRICTION PATTERNS OF DNA, Lorne A. Babiuk, Robert M. Blumenthal and Vikram Misra Dept. Vet. Microbiology, WCVM, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0

Bovine herpesviruses (types 1, 2, and 3) have been subjected to both O'Farrell two-dimensional analysis of virion proteins and restriction enzyme analysis of the viral DNA in an attempt to determine the genetic relationship between the three herpesvirus types and the degree of variation within each type. This approach allows a preliminary correlation between specific proteins and/or restriction fragments and the tropism of the herpesvirus type, which may be useful in future epidemiological studies. Individual isolates were found to be quite stable, showing no detectible changes over several passages in either cows (following reactivation of the latent virus) or tissue cultures. Differences between isotypic isolates suggest that certain loci may have a comparatively high proclivity for successful mutation. The proteins having the greatest apparent variation among the isolates are being analyzed as to their location and function in the virion.

725 NONSENSE: THE STUDY OF MUTATIONS IN THE HSV-1 THYMIDINE KINASE GENE. Wilma P. Summers and William C. Summers, Yale University School of Medicine, New Haven, CT. 06510

We are characterizing our collection of HSV-1 thymidine kinase mutants with respect to several properties. Of special interest are those which are nonsense mutants as shown by their suppressibility in vitro with yeast suppressor tRNAs. These mutants are currently being used to study ambiguity suppression induced by the antibiotic paromomycin. In addition, several schemes for selection of cells carrying nonsense suppressors are under way.

We have refined the use of the ^{125}Iodeoxycytidine assay for HSV-specific TK and find it useful for characterizing a) the reversion frequency of TK$^-$ mutants, b) low level activity mutants, c) in vitro suppressibility, and d) the presence of HSV TK in biochemically transformed cells.

726 GENETIC AND PHYSICAL STUDIES OF THE THYMIDINE KINASE GENE OF HERPES SIMPLEX VIRUS. Michael J. Wagner, James R. Smiley, and William C. Summers, Yale University, New Haven, Conn. 06520

We have constructed genetic and physical maps of the thymidine kinase gene of herpes simplex virus type 1. These maps can be aligned with both the genetic and physical maps of the entire HSV-1 genome. The molecular weights of the TK-related polypeptides produced by TK mutants (including one known chain termination mutant) correlate with the positions of the mutations on the genetic map, which implies a leftward direction of transcription of the TK gene on the prototype arrangement of the HSV genome. This direction of transcription was confirmed by hybridization of TK mRNA to the separated strands of a cloned BamHI fragment of HSV-1 DNA which contains the TK gene.

We are currently determining the complete nucleotide sequence of the HSV-1 thymidine kinase gene. EcoRI fragments of the cloned TK gene were subcloned into the single-stranded bacteriophage M13MP2 to be used as templates in the Sanger dideoxy sequencing method. Using this method, we have obtained the nucleotide sequence of the amino terminal end of the TK gene, including a possible site for initiation of transcription.

727 GROWTH INHIBITION BY ACYCLOGUANOSINE (ACG) OF HERPES SIMPLEX VIRUS (HSV) IN DIFFERENT CELL STRAINS, Johan Harmenberg, Dept. Virol., Nat. Bact. Lab., S-105 21 Stockholm, Sweden

ACG, which is phosphorylated by HSV induced thymidine kinase (TK), inhibits HSV DNA-polymerase (Elion et al., 1977). The inhibition of plaque formation by HSV-1 was studied in HL (human lung fibroblasts), HeLa (human cervical carcinoma), Sirc (rabbit cornea), RD (human rhabdomyosarcoma) and GMK (green monkey kidney) cells. The 50 % plaque reduction does (PR_{50}) in HL cells was 0.3 µM ACG, in HeLa, Sirc and RD cells it was 1.5, 1.75 and 1.75 µM ACG respectively. HSV-1 infected GMK cells were relatively insensitive to ACG inhibition, with PR_{50} of 60 µM ACG. HSV-1 plaque reduction on HL cells was studied as a function of increasing virus concentrations with constant ACG. At 2.5 µM ACG the plaque reduction was 75, 60, 26% at m.o.i.'s of 0.0001, 0.001, 0.01. ACG at concentrations of 200 µM or less, do not significantly effect host cell DNA-synthesis, as measured by ^{3}H-thymidine incorporation.

In human lung fibroblasts, the viral plaque formation by HSV-1 was inhibited by a very low ACG concentration. The cellular TK activity may influence the rate of ACG phosphorylation, and thus increase the specific antiviral activity.

728 EXPRESSION OF A DEFINED REGION OF THE HSV-1 GENOME, Kevin P. Anderson and Edward K. Wagner, University of California, Irvine, Irvine, CA 92717.

We are currently studying the molecular events resulting in expression of certain defined regions of the HSV-1 genome. One such region is that encompassed by HindIII fragment K (0.53-0.59 from the left end of the prototype arrangement of HSV-1). This region is marginally expressed in the absence of de novo protein synthesis, and prior to viral DNA synthesis this region encodes a single major RNA species 5.2 kb in size. Additional mRNA species (7.0 kb, 3.8 kb, 1.8 kb and 1.5 kb) complementary to this region of the viral genome are present following the onset of viral DNA replication. These polyadenylated messages have been isolated from polyribosomes of infected cells by preparative hybridization to HindIII fragment K DNA covalently coupled to cellulose and the location of the 3' ends and the in vitro translation products of each of them have been determined. The 7, 5.2, and at least one of the mRNAs smaller than 2 kb are transcribed in the same direction and have 3' ends mapping in the region 0.59 to 0.61. Thus, these messages are at least partially colinear. Analysis of the polypeptides translated from the HindIII K specific mRNAs in vitro shows that two mRNAs, the 7 kb and one less than 2 kb, encode the same polypeptide. The other mRNA species encode discrete polypeptides. HindIII fragment K specific RNA isolated from the nucleus of infected cells late after infection contains species as large as 10 kb in size. We are currently engaged in a detailed characterization of these putative RNA precursors.

729 DETERMINATION OF THE "SENSE" STRAND OF THE HERPES SIMPLEX I THYMIDINE KINASE GENE, David L. Hare, Lewis I. Pizer, and John R. Sadler, University of Colorado Health Sciences Center, Denver, CO 80262

A 2.4 kb Eco RI fragment containing the majority of the coding sequence for the Herpes Simplex I thymidine kinase gene was cloned into an E. coli EK1 vector. The strands of the vector were separated, labeled in vitro, and each strand hybridized to total cytoplasmic RNA isolated from HSV I infected cells. Results indicated that more of the RNA hybridized with that DNA having its 3' end nearest the internal Hind II site, suggesting that this DNA strand is the "sense" strand for the HSV I thymidine kinase gene. However, since a significant proportion of RNA bound to the other DNA strand, it is likely that portions of this region are transcribed from both strands and are transported into the cytoplasm.

730 GENETICS OF ACYCLOGUANOSINE RESISTANCE AND THE THYMIDINE KINASE GENE IN HSV-1, Donald Coen and Priscilla Schaffer, Sidney Farber Cancer Institute, Boston, MA 02115

We have studied the genetics of acycloguanosine (ACG) resistance in HSV-1, strain KOS. Nearly all ACG-resistant mutants examined exhibited reduced levels of HSV-specific thymidine kinase (TK) activity. In general, the lower the TK activity, the greater the degree of ACG-resistance. Moreover, a mutant was isolated which was ACG-resistant at 39° and ACG-sensitive at 34° and which induced thermolabile TK activity. This class of ACG-resistant mutants, which we term ACG^r-TK, maps to one locus between the A and G loci on the KOS linkage map. This location corresponds well with the known physical map location of the TK gene and the A and G loci. ACG^r-TK mutations are recessive with respect to the wild-type phenotype (ACG^s). We are examining the fine structure of this genetic locus with the aid of a cloned DNA fragment bearing the KOS TK gene.

A second locus for ACG-resistance was identified in the mutant PAA^r5 which was derived by passage of KOS in phosphonoacetic acid (PAA). This mutant induces wild-type levels of TK activity, yet is resistant to ACG. Its ACG^r locus, which we term ACG^r-PAA, is separable by recombination from ACG^r-TK mutations. The ACG-resistance of this mutant, unlike ACG^r-TK mutants, behaves as though it were co-dominant with the wild-type phenotype. The two types of ACG^r mutants complement producing drug-sensitive gene products resulting in growth inhibition in ACG. The ACG^r-PAA locus in PAA^r5 is linked to the locus for PAA-resistance suggesting that resistance to both drugs arose from a single mutation in the viral DNA polymerase gene.

731 THE ROLE OF AN IMMEDIATE-EARLY VIRAL GENE IN THE REGULATION OF HSV-1 GENE EXPRESSION, R.A.F. Dixon, R.J. Courtney*, and P.A. Schaffer, The Sidney Farber Cancer Inst., Harvard Medical School, Boston, MA 02115, *Dept. of Microbiology, Univ. of Tennessee, Knoxville, TN.

Herpes simplex virus (HSV) specific proteins fall into at least three kinetic classes whose synthesis is sequentially and coordinately regulated. Temperature-sensitive mutants in one cistron (1-2) are defective in the transition from immediate-early to early and late synthesis. In order to elucidate the function of the 1-2 gene, a series of 9 ts mutants in this cistron have been mapped fine structurally and characterized biochemically.

Physical mapping by homotypic marker rescue has shown that all members of the group lie within the terminal repeat sequences of the S region of the genome. Fine structure genetic and physical mapping has permitted the mutants to be ordered and separated within the repeat. Because it has been shown that the message for VP175 and the DNA template specifying this protein extend beyond the limits of the physical map of the mutants, it follows that they must all lie within the structural gene for VP175.

SDS-PAGE analysis has shown that all members of the group overproduce the immediate-early proteins VP175, 136, 110 and 63, and markedly underproduce early and late proteins at the nonpermissive temperature. In temperature shift-up experiments, it was found that the synthesis of early and late proteins ceased, while the synthesis of immediate-early proteins began again. Thus, it is postulated that VP175 is a) involved in the transition from immediate-early to early protein synthesis, b) required continuously to maintain early protein synthesis, and c) autoregulated, acting to inhibit immediate-early protein synthesis.

732 ALTERED GLYCOPROTEIN SYNTHESIS BY A Ts MUTANT OF HERPES SIMPLEX VIRUS WHICH PRODUCES ENVELOPED BUT NONINFECTIOUS PARTICLES, S.P. Little[1], J. Jofre[2], R.J. Courtney[3] and P.A. Schaffer[1]. [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA., [2]Department of Microbiology, University of Barcelona, Barcelona, Spain, [3]Department of Microbiology, University of Tennessee, Knoxville, Tenn. 37916.

A temperature-sensitive (ts) mutant of herpes simplex virus type 1 (HSV-1), ts J12, is able to undergo one cycle of replication at the nonpermissive temperature (39°) yielding wild-type quantities of mature virus particles. Although they are not infectious, these particles contain viral DNA which is as infectious as wild-type virus DNA. Thus, a component of the mutant virion appears to be responsible for the lack of infectivity of these particles.

One and two dimensional polyacrylamide gel electrophoresis of ^{14}C-glucosamine-labelled extracts demonstrated that ts J12 synthesizes an abberrant gB glycoprotein which migrates at a lower molecular weight than the analogous wild-type species both at 34° and 39°. The gB glycoprotein has been reported to be essential for virion penetration and to inhibit the formation of syncitia (Manservigi et al., PNAS 74: 3913 (1977), Sarmiento et al. J. Virol. 29: 1149 (1979)). That the functional consequences of the defect in gB exhibited by ts J12 is manifested at 39° but not at 34° is evident from the fact that virions produced at 34° are able to penetrate cells but those produced at 39° are not. Furthermore, the defect in gB at 34° is apparently insufficient to effect its ability to inhibit syncitia formation, as all ts J12 plaques at 34° are nonsyncitial. Consistent with the phenotypic properties of ts J12 is the observation that the ts defect in this mutant lies within the limits of the DNA sequences which specify gB on the physical map of the genome.

733 ISOLATION AND PRELIMINARY CHARACTERIZATION OF HERPES SIMPLEX VIRUS MUTANTS RESISTANT TO IMMUNE CYTOLYSIS. B.A.Pancake[1], N.A.Machtiger[1], R.E.Eberle[2], R.J.Courtney[2], S.S.Tevethia[3], and P.A.Schaffer[1]. [1]Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, [2]Department of Microbiology, University of Tennessee, Knoxville, TN 37916, and [3]Department of Microbiology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

Immune cytolysis mediated by antibody and complement is directed against components of the major herpes simplex virus (HSV) glycoprotein complex (gABC, MW 115-130,000) present on the surface of infected cells. In order to investigate the role of viral glycoproteins in the biology of HSV, we have developed a procedure for the selective enrichment of HSV mutants defective in the expression of specific glycoproteins at the cell surface, using antisera directed against individual viral glycoproteins (anti-gABC, -gAB, and -gC) in the immune cytolysis test. Cells infected with mutagenized wild-type virus and incubated at 40°C for 18 hr. were subjected to immune cytolysis using glycoprotein-specific antisera. Surviving infectious centers were plated at 34°C and plaques were picked. Seventy-three mutants exhibiting temperature-sensitivity (*ts*) for growth and/or altered cytopathic effects (*cpe*) were obtained. Cells infected with representative mutants of both types (*ts* and *cpe*) were markedly resistant to cytolysis at 34° and/or 40°C compared with the results using wild-type virus. Complementation analysis with *ts* mutants indicates that two or more gene functions can affect the expression of the gC glycoprotein, while at least five essential functions can affect the expression of the antigenically related gA and gB glycoproteins.

734 A GENE FUNCTION OF HERPES SIMPLEX VIRUS REQUIRED FOR EXPRESSION OF ALL EARLY VIRAL GENE PRODUCTS. David M. Knipe, William Batterson, and Bernard Roizman. Univ. of Chicago, Chicago, IL 60637

The mutant HSV-1 (HFEM) tsB7 fails to express any detectable viral polypeptides and fails to inhibit host cell protein synthesis in infected cells maintained at the non-permissive temperature. The mutant can complement the growth of other viruses and therefore can enter doubly infected cells. The mutation maps in the middle of the L component in or near genes coding for virion structural proteins and near a previously mapped function regulating inhibition of host cell protein synthesis. Abundant transcripts and mRNA from this region accumulate only after DNA synthesis and thus the gene appears to code for a late protein. All of our data are consistent with the lesion being a defect in a virion structural protein required for expression of all early viral gene products and for inhibition of host cell protein synthesis. This mutation defines a new early stage of infection prior to expression of any viral gene products at which the replication of herpes simplex virus can be regulated.

735 MOLECULAR CLONING OF THE HUMAN CYTOMEGALOVIRUS GENOME (STRAIN AD169), Joyce C. Tamashiro and Deborah H. Spector, University of California, San Diego, La Jolla, CA 92093

Human cytomegalovirus (HCMV), one of the herpesviruses, is medically significant both as a cause of birth defects and as a source of problems in immunosuppressed individuals. Because the virus is highly cell-associated, it is difficult to obtain large quantities of pure viral DNA. For this reason, we have sought to construct a cloned library of the HCMV genome to aid in further studies of the molecular biology of HCMV infections.

The cloning was accomplished as follows: Eco RI fragments of the AD169 strain of HCMV (0.27 ug of DNA) were inserted into the Eco RI site of the E. coli plasmid PACYC184. Approximately 9,000 colonies were selected on the basis of tetracycline resistance and chloramphenicol sensitivity. After isolation and Eco RI cleavage of the recombinant plasmids, the cloned fragments were analyzed for co-migration on agarose gels with authentic Eco RI digested HCMV DNA. The viral origin of the fragments was determined by hybridization of ^{32}P HCMV DNA to Southern blots of the inserts. To date, we have analyzed 80 clones and the viral inserts obtained represent at least 70% of the genome. We are currently constructing a physical map of the genome for ordering the fragments.

736 INHIBITION AND INDUCTION OF CMV LATE ANTIGENS DURING AND AFTER PFA, Britta Wahren and Bo Öberg, Department of Virology, Karolinska Institute S-105 21 Stockholm and Astra Läkemedel AB, Södertälje, Sweden.

Cellular and viral antigens appearing after human CMV infection were studied in the presence of phosphonoformate (PFA). Complete inhibition of CMV replication was obtained at 500 µM, 50% plaque reduction at 100 µM PFA. Early nuclear CMV antigens (EA) were not inhibited, but the formation of nuclear inclusion bodies, late cytoplasmic antigens (LA), Fc-receptors and cytopathic effects were inhibited by PFA.

By microimmunofluorometry, antigens of cells in different stages of CMV infection were quantified. In naturally aborted infection, the largest amount of EA was seen between days 3-5. In PFA treated cells, high EA levels persisted up to day 14, by 35 days it was hardly measurable. Late antigens and complete infectious virus appeared delayed after PFA had been deleted. It seems possible to induce an artificial latent CMV infection of all infected cells by the use of PFA.

737 EVIDENCE FOR INVERTED REPETITIONS, TERMINAL HETEROGENEITY AND FOUR ISOMERIC FORMS OF HUMAN CYTOMEGALOVIRUS DNA, R. LaFemina and G.S. Hayward, Dept. of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Infections with human cytomegalovirus (HCMV), a member of the herpes virus family, are of serious clinical concern especially as cytomegalic inclusion disease in the newborn and in immunosuppressed renal transplant patients. Inactivated virus has the ability to transform cells in vitro. We have been interested in the construction of restriction enzyme maps for HCMV strain Towne DNA as a prelude to the localization of specific gene functions. Electron microscopy of self annealed single strands suggests that the HCMV genome can be divided into long (L) and short (S) unique regions each bounded by inverted terminal repetitions. Digestion of Towne DNA with BamHI, BclI, BglII, EcoRI, HindIII and XbaI reveals the presence of submolar end and L-S joint fragments suggesting the existence of 4 structural isomers. The terminal fragments obtained after restriction enzyme digestion have been identified by the disappearance of submolar end fragments upon prior treatment with λ5' exonuclease. The hybridization pattern of specific fragments mapped at the L repeat-unique junction defines the size of the L repeat, while the use of S and L end probes confirms the existence of 4 structural isomers. The L repeats of 12 kilobases (kb) bound the L unique region of 170 kb while the unique S region of 36 kb is bounded by the 2 kb S repeats. The L repeat-unique junction may be the site of strain variation; fragments mapped at this location for strain Towne are shorter or missing in strain Davis. As opposed to the L end terminal heterogeneity seen in herpes simplex, HCMV displays split end heterogeneity predominently around the S end. Maps of cleavage sites for HindIII and XbaI have been completed for the entire 235kb genome.

738 IN VITRO ACTIVATION OF HUMAN CYTOMEGALOVIRUS. H. Gadler[+] and C.-G. Groth[x], [+]Dept. of Virol., Nat. Bact. Lab., S-105 21 Stockholm; [x]Transpl. Unit., Huddinge Hosp., S-141 86 Huddinge, Sweden.

In order to increase the understanding of interactions between herpesviruses and cells, latent viral infections are studied. We have studied the in vitro activation of human cytomegalovirus (CMV) from blood cells of immunosuppressed renal transplant patients. The blood was separated into a mononuclear, a polymorphonuclear and a red blood cell fraction. CMV could be isolated from blood cells from 44%. Most commonly the polymorphonuclear cell fraction contained virus, 38%. The mononuclear cell fraction yielded virus in 25%. The antibody activity in complement fixation (CF) and the presence of antibodies against early viral antigens in immunofluorescence (CMV-EA) has been investigated. Antibodies against CMV-EA did regularly appear and reached high titers but decreased more rapidly than CF antibody. CMV isolation from blood cells seems to be a common phenomenon in renal transplant patients. Leukocytes may be one of the sources of latent CMV albeit harbouring it in a low frequency.

739 GENETIC MAPPING OF VERY LARGE DNA MOLECULES: AN ELECTRON MICROSCOPIC APPROACH, Claire Moore and Jack Griffith, Cancer Research Center, University of North Carolina, Chapel Hill, N.C. 27514

Physical mapping of the Herpes virus genomes through the use of restriction enzymes has proven time-consuming due to the very large size of the DNA molecules and to the presence of sequence isomers. We have developed a means of directly visualizing sites of restriction enzyme cleavage on uncut DNA using the electron microscope. With classic surface spreading microscopy, potential sites of cleavage of a restriction enzyme (to which specific tags had been synthesized) are made visible on the intact DNA by the presence of small marker RNA molecules. This technique provides a way of direct restriction mapping with very large and heterogeneous DNAs, and requires only small quantities of DNA, an advantage in systems such as the non-producing EBV lines which have only a few copies of the viral genome per cell. Furthermore, isolated RNA transcripts may be included in the mapping procedure and their location identified relative to nearby restriction sites. Details of this method, in which an aliquot of the DNA is cut, digested with Exonuclease III, treated with RNA polymerase, and the resulting small RNAs isolated and used as tags, will be described, as well as initial applications of mapping of EBV and other Herpes group viruses.

740 Identification of Terminal Fragments of Herpesvirus papio DNA (HVP) and its Relationship to Epstein-Barr Virus DNA. Y.S. Lee, A. Tanaka, R. Y. Lau, M. Nonoyama, Life Sciences, Inc. St. Petersburg, FL 33710 and H. Rabin, Frederick Cancer Research Center, Frederick, MD 21701.

An EBV-like herpesvirus, HVP, has been isolated from a baboon lymphoblastoid cell line (594S/F9), induced by the tumor promotor 12-O-tetra-decanoylphorbol-13-acetate (TPA). The HVP DNA was isolated from purified virions and digested with restriction endonucleases (EcoRI, Hind III, Bam HI, Pvu I and Sal I). The fragments were separated in 0.4% agarose gel by electrophoresis. The number of bands produced by each enzyme was as follows: 12 for EcoRI, 12 for Hind III, 15 for Bam HI, 4 for Pvu I and 10 for Sal I. The total molecular weight of the fragments ranges from 94 to 107 x 10^6 daltons.

To identify the terminal fragments of the DNA molecules, HVP DNA was treated with λ exonuclease prior to endonuclease digestion. EcoRI A and J fragments and Hind III A and H fragments were found to be sensitive to λ exonuclease. Furthermore, Southern hybridization showed that the EcoRI A fragment hybridized to the Hind III H fragment and EcoRI J to Hind III A, indicating that EcoRI A and Hind III H fragments are located on one end of the molecule and EcoRI J and Hind III A on the other end of the molecule. The terminal fragments of B95 DNA cleaved with EcoRI or Hind III were also compared with those of HVP DNA cleaved with EcoRI. The EcoRI A fragment of 594S/F9 DNA was hybridized to the Hind III D fragment and to the EcoRI C and D fragments of B95 DNA. This indicates that the topographical relationship between HVP and EBV DNA is at least found in the terminal fragments. The preliminary physical mapping data of HVP DNA are discussed.

741 ORGANIZATION AND EXPRESSION OF THE VACCINIA VIRUS GENOME, Riccardo Wittek, Jonathan A. Cooper, and Bernard Moss, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, 20205

Information concerning the organization and expression of the vaccinia virus genome has been obtained using DNA fragments cloned in coliphage lambda. Starting at the left-end of the genome, the cloned fragments comprise a continuous 20,500 nucleotide base-pair stretch of DNA, the terminal half of which is repeated at the right end of the genome. RNAs encoded by the region of interest have been resolved by agarose gel electrophoresis, immobilized on paper, and mapped using ^{32}P-labeled DNA restriction fragments. Early in infection, discrete areas of the DNA are transcribed to produce RNAs of 0.5 to 1.3 Kb which can be translated in vitro. Three early mRNAs and their translation products have been mapped within the repeated region and seven within the unique. Of interest is the apparent lack of transcription from the terminal 3,000 nucleotide base pairs of the genome. Although both strands of the DNA are transcribed early in infection, the majority are encoded within the leftward-reading strand. Additional higher molecular weight RNAs have been detected and mapped by the above blotting technique as well as by gel electrophoretic analysis of nuclease S1-resistant hybrids formed by annealing of RNA to ^{32}P-labeled DNA strands. The cloned region of the genome appears to encode few mRNAs for specific late proteins. One of them has been mapped and appears to be quite heterogeneous in length. Additional data suggest that this heterogeneity may be a common property of many late mRNAs. Models for the generation of high molecular weight and variable length RNAs will be discussed.

742 GENE ORGANIZATION OF A BACULOVIRUS, Lois K. Miller, Dept. of Bacteriology and Biochemistry, The University of Idaho, Moscow, ID 83843

Baculoviruses possess large, circular, covalently-closed, double-stranded DNA genomes and replicate in the nuclei of invertebrate host cells. The viruses undergo a complex process of development which involves both the formation of nonoccluded virus by budding through cellular membranes and the formation of occluded virus within the nucleus. Non-occluded virus is the infectious agent in cell to cell transmission whereas occluded virus effects transmission at the organism level. My laboratory has isolated a series of temperature sensitive (ts) mutants of Autographa californica nuclear polyhedrosis virus (AcMNPV), a model baculovirus with a broad host range (Lee and Miller, 1979, J. Virol. 31:240-252). A number of these mutants are ts for occlusion but are not ts for nonoccluded virus formation. We have constructed a restriction endonuclease fragment map of the AcMNPV DNA genome (Miller and Dawes, 1979, J. Virol. 29:1044-1055) and are currently constructing a genetic map by marker rescue of mutant genomes with wild-type restriction fragments. In the case of viruses ts for occlusion, marker rescue involves distinguishing plaques with and without occluded viruses. In addition to their basic virological interest, baculoviruses are currently the only viruses with potential as vectors for transducing invertebrate cells and a knowledge of gene organization will be valuable from this perspective. The region controlling occlusion should be an excellent site for inserting passenger DNA since the virus will be defective in transmission in nature.

743 MOLECULAR GENETICS OF THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE, Greg Reyes and G.S. Hayward, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Both herpes simplex virus types 1 and 2 specify a deoxypyrimidine kinase enzyme (TK). Selective (HAT media) and counterselective (BUdR) measures exist to obtain cells that express or suppress the expression of this distinctive viral gene. The herpes TK system therefore allows investigation of the viral and host interactions involved in gene expression. The minimal coding region for viral TK has been determined to 1% of the 150 Kb genome (0.303-0.314 fractional map units) by utilizing restriction enzyme cleavage and transfection into Ltk^- cells. Multiple TK^+ cell lines that contain various amounts of viral specific information, in addition to the HSV-2 TK gene, have been isolated by transfection of DNA fragments generated with different restriction enzymes (HindIII, BglII, SalI, and BstEII). The TK activity of these various HSV-2 TK^+ cell lines has been characterized as viral specific by the use of the thymidine analog arabinosylthymine, and the presence of viral DNA has been confirmed by Southern blot analysis. We have also obtained data by Southern blot hybridization and hybridization kinetics which indicate a multiple (~10) copy insert, tandemly arranged in two TK^+ cell lines generated by transfecting the XbaI-D and BamHI-O fragments of HSV-1. The SalI-G fragment (5.6Kb) containing HSV-2 TK has been cloned in pBR322. The role of this recombinant as a secondary cloning vehicle useful for cotransfection procedures (i.e., the incorporation into the celluar genome of unselected genes linked to HSV-2 TK) will be discussed. Experiments aimed at obtaining new virus host range mutants which grow only on TK^+ cell lines containing viral sequences normally adjacent to the TK gene, or transposed into an adjacent position within recombinant plasmids, are in progress.

744 DNA ANALYSIS OF DIFFERENT EPSTEIN-BARR VIRUS STRAINS, Georg W. Bornkamm and Hajo Delius, Institut für Virologie, Freiburg, West Germany

EBV DNA originating from patients with Burkitt's Lymphoma (BL), Nasopharyngeal Carcinoma (NPC), infectious Mononucleosis and a patient with acute lymphoblastic leukemia (ALL) was compared by partial denaturation analysis and by extending Given and Kieff's restriction enzyme data of the strains B95-8 and W91 to the other isolates. In addition to these strains viral DNA from the following cell lines was studied: P3HR-1, a human cell line of BL origin producing a non-transforming virus; M-ABA, a marmoset cell line transformed by a NPC derived virus; QIMR-WIL, a human producer cell line from a patient with ALL and Raji, a human BL derived nonproducer cell line, from which episomal viral DNA was isolated.

The partial denaturation and restriction enzyme maps of the different strains show great similarities and do not favor the idea, that different viral strains are associated with different diseases. All strains showed variations in the number of internal repeats, the number of repeats being not even constant and stable within one viral strain. Adjacent to the internal repeats a sequence carrying a HindIII site is deleted in the nontransforming P3HR-1 strain. This region of the genome will be preferentially analyzed for transforming genes. Additional variations between different strains were observed in the HindIII D_2 and the HindIII E fragments at the right hand site of the EBV genome.

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745 CHARACTERIZATION OF SMALL RNAS ISOLATED FROM VESICULAR STOMATITIS VIRUS AND DEFECTIVE INTERFERING PARTICLES, Cheryl L. Isaac and Jack D. Keene, Department of Microbiology and Immunology, Duke University Medical Center, Durham, North Carolina 27710

We have examined total RNA extracted from highly purified VSV and DI particles for species smaller than 42s RNA. After terminal labeling and separation on urea-acrylamide gels, the predominant small RNAs are 4s in size. Very little 2s RNA resembling leader or DI particle product was found. By several criteria the 4s RNAs appear to be transfer RNA. They contain 3' ACC and upon analysis by one and two dimensional gel electrophoresis only minor differences in patterns were noted when 4s RNAs from VSV and DIP were compared to total BHK host tRNA. Furthermore, no singly predominant species of tRNA was apparent among the viral 4s RNAs. Utilizing terminal labels and beta-elimination of separated species of viral 4s RNA, strand scissions were found where methylated bases characteristically occur in mammalian transfer RNA. Transfer RNAs are known to be packaged by RNA tumor viruses but we have not found 4s RNA in reovirus, thus suggesting an association of tRNA with enveloped viruses.

746 A VARIANT VSV WITH ALTERED TRANSCRIPTION PROCESSING AND TERMINATION FUNCTIONS IN VITRO, Jacques Perrault, Jeri Lane, and Marcella M. McClure, Washington University, St. Louis, MO 63110.

We have isolated a variant of vesicular stomatitis virus (VSV) after multiple cycles of heat inactivation and growth of survivors, which gives rise to altered transcription products in vitro as compared to wild-type VSV. Infectious virions of this variant, designated polR1, synthesize a broad size range of RNA products, few of which correspond to the electrophoretic mobility of wild-type mRNAs. The differences between wild-type and polR1 are however most striking when comparing in vitro RNA products from defective interfering particles (DI) generated from these. Whereas wild-type DI particle polymerase copies the 3' end of the template until residue 46, the polR1 polymerase apparently reads past this termination site and synthesizes a nearly complete copy of the template. The variant polymerase also appears able to copy wild-type DI RNA templates which have been packaged by polR1 specified proteins. We propose that the polR1 mutation(s) affects the regulation of transcription versus replication functions of the VSV coded polymerase.

747 ANALYSIS OF TRANSCRIPTION ATTENUATION ON THE VSV GENOME, Linda E. Iverson and John K. Rose, Tumor Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, California 92138

We are examining why the five separate mRNA species of vesicular stomatitis virus (VSV) are produced in decreasing amounts as transcription proceeds along the single-stranded RNA genome. To study this transcription attenuation we have identified recombinant plasmids containing sequences derived from the 5' and 3' ends of four of the five VSV mRNAs (N, NS, M and G). We have used these plasmids in DNA excess filter hybridizations to study the amount of transcription occurring at both ends of each gene. Our results confirm and extend an earlier report of decreasing molar yields of each mRNA species corresponding to the gene order (N-NS-M-G-L) and also confirm the sequential nature of VSV transcription. Additional experiments suggest that transcription attenuation is predominantly localized to the gene termini or the intergenic regions.

748 ANALYSIS OF VSV GENE, INTERGENE, AND GLYCOPROTEIN STRUCTURE USING RECOMBINANT PLASMIDS, John K. Rose, Tumor Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, California 92138

Plasmids containing vesicular stomatitis virus (VSV) mRNA sequences were used to 1) obtain sequences corresponding to the 3'-termini of VSV mRNAs and 2) generate primers that were used to sequence through the genomic RNA regions joining the NS, M, G and L genes. Together with previous results, these results provide the complete set of VSV intergenic and flanking gene sequences. Extensive homologies were found among the four junctions of the five VSV genes. These regions have the common structure, (3')AUACUUUUUUUNAUUGUCNNUAG(5') in which N indicates three variable positions in the 23 nucleotide sequence. The first eleven nucleotides of this sequence are complementary to the sequence (5')UAUGAAAAAAA(3') which occurs at the mRNA-poly(A) junction in each mRNA. These sequences presumably signal polyadenylation of each mRNA. Dinucleotide spacers (CA or GA) whose complements do not appear in the mRNAs, follow the polyadenylation signals and constitute the intergenic regions. Immediately after these dinucleotides are the sequences complementary to the 5'-terminal sequences on each mRNA.

The amino acid sequence of the COOH-terminus of the VSV glycoprotein was determined from the sequence of a recombinant plasmid. It contains a sequence of 20 hydrophobic residues flanked by basic residues. This sequence probably anchors the protein in the viral membrane.

749 NUCLEOTIDE SEQUENCES OF T1 RESISTANT OLIGONUCLEOTIDES OF VSV STANDARD AND DI RNA, Frederick S. Hagen and Alice S. Huang, Children's Hospital Medical Center and Harvard Medical School, Boston, MA 02115.

The RNA of standard vesicular stomatitis virus (VSV, San Juan) and two defective interfering (DI) viruses, DI 0.52 and DI-T, have been fingerprinted and the major T1-resistant oligonucleotides sequenced. Most of the oligonucleotides of the DI RNAs are shared by the standard RNA. In addition, the DI-T RNA has two oligonucleotides and DI 0.52 has one oligonucleotide which are unique. One unique oligonucleotide of DI-T, 3' GUCUAUUUUUUAUUUUU 5', is a sequence from the 3' end region of the DI RNA. An oligonucleotide number 21 5'AUAAAAAAUAAAAACCACAAG 3' shared by all the RNAs is from the 5' ends and is complementary to sequences found in the 3' end of the RNA in DI-T. The second unique oligonucleotide of DI-T and the only unique oligonucleotide of DI 0.52 differ by only one nucleotide change (a C to U change and a U to A change) from oligonucleotides 12 and 17, respectively, of standard RNA. These data indicate that the genomes of DI viruses are not simple deletions, but contain, in addition, complementary sequences and point mutations.

750 DI PARTICLE MEDIATED INTERFERENCE OF VESICULAR STOMATITIS VIRUS. Frank M. Horodyski, Bert L. Semler and John J. Holland, Department of Biology, University of California, San Diego, La Jolla, Ca. 92093

Defective interfering (DI) particles of vesicular stomatitis virus (VSV) interfere specifically with replication of homologous standard virus. A quantitative assay which measures the yield of progeny standard virus and DI particles was employed to investigate this interference property. The UV inactivation kinetics of MST, a DI particle derived from the 5' end of the VSV genome, was seen to follow one-hit kinetics with a target size equal to the RNA genome size of this DI particle. We determined that it is possible to render the DI particle unable to replicate while still being able to synthesize small RNA *in vitro* by irradiating DI particles with a higher UV dose. Even when a very high input multiplicity of such DI particles treated with this UV dose is used, no effect of these DI particles was seen, neither in production of infectious virus nor in total intracellular viral specific RNA synthesis. These results indicate that replication of the entire DI particle RNA, and not merely synthesis of small RNA is necessary for interference to occur in this system. The interference property was also studied using DI particles which were originally used to establish a persistent infecton of VSV in BHK_{21} cells, and standard virus which was recovered from the persistent infection at various times. The interfering ability of this DI particle was vastly different depending which helper virus was used. These preliminary results suggest a possible role of DI particles in selecting virus mutants during persistent infection.

751 DI PARTICLES PROTECT GENETICALLY SUSCEPTIBLE HAMSTERS FROM LETHAL VSV INFECTION P.N.Fultz, C.-Y.Kang, J.Shadduck, M.J.Nelles, J.W.Streilein; UTHSCD,Dallas,Texas 75235

Syrian hamsters exhibit an extremely high mortality rate if infected with large doses (10^8 PFU) of vesicular stomatitis virus (VSV)-a dose not lethal to mice via any route of injection except intracranial. VSV-infected hamsters died within three days; histopathologic study revealed multifocal hepatic necrosis and necrosis of splenic lymphoid tissue. Animals from six genetically defined strains were tested for susceptibility to low doses of VSV by intraperitoneal injection of from 10^1 to 10^4 PFU per animal. Mortality at seven days indicated that the six strains could be classified into three groups according to resistance: (1) LSH,MHA-16% survived; (2) CB,MIT,UT2-50 to 75% survived; (3) UT1-92% survived. Group 1 hamsters were killed with as few as 10 PFU, while only a small number of animals in the remaining groups succumbed to this dose. Random bred LGV hamsters exhibited an LD_{50} with VSV of 10^5 PFU. Thus, resistance to lethal VSV infection in hamsters is genetically determined.

Defective interfering (DI) particles may play an important role in host defense against systemic viral infection. To test this hypothesis, susceptible LSH hamsters were simultaneously injected with a lethal dose of VSV (10^4 PFU) and graded amounts of homologous DI particles (10^6 to 10^{10}), *i.e.*, ratios of DI particles to PFU of 10^2:1 to 10^6:1. Significant protection (63% survived 10^4 PFU of VSV) was seen at a DI to VSV ratio of 10^3:1 or greater. These data indicate that hamsters can be effectively protected from a lethal infection of VSV by DI particles. With this system, we can examine the possible correlation between the generation of DI particles by host cells and the genetic basis of *in vivo* host resistance to VSV.

752 VIRUS ISOLATED FROM BHK CELLS PERSISTENTLY INFECTED FOR MANY YEARS WITH VSV. Katherine R. Spindler and John Holland, Univ. of California, San Diego, La Jolla, CA.

Vesicular stomatitis virus (VSV) isolated following more than 5 1/2 years of persistent infection of BHK_{21} cells *in vitro* has become increasingly debilitated. Recovered virus has undergone massive genome mutation relative to the tsG31 virus originally used to establish persistence, as shown by oligonucleotide and peptide mapping. The 70 month virus initially had a particle/infectivity ratio that was 2-3 orders of magnitude higher than that of tsG31 or of virus isolated at 5 years of persistent infection (60 month virus). During successive passages of a clonal pool of this isolated 70 month virus, the virus titers increased 300-1000 fold at each passage, until after five successive passages, titers comparable to those of tsG31 were obtained. Due to some (perhaps many) of the accumulated genome mutations, cloned virus isolated after 69 months of persistent infection has gained the ability to establish a new persistent infection in the absence of added defective interfering (DI) particles. This virus did cause severe cytopathology in BHK_{21} cells, but after recovering from the initial cytopathology the cells established stable carrier cultures. These carrier cultures shed DI particles and are in all other ways characteristic of persistently infected cells. 60 and 70 month virus were not able to cause disease, nor to kill young mice when injected intracerebrally. This is in contrast to the tsG31, which at high moi kills nearly all mice injected, although more slowly than wild type (Mudd-Summers) VSV. We are further characterizing the biochemical and biological differences observed in these virus isolated after nearly 6 years of persistent infection to better understand the events and mechanisms of long term persistent infection by virulent RNA viruses.

753 IN VITRO SYNTHESIS OF THE FULL-LENGTH COMPLEMENT OF DEFECTIVE INTERFERING PARTICLE RNA OF VESICULAR STOMATITIS VIRUS, Amiya K. Banerjee[1], Pranab K. Chanda[1], and C. Yong Kang[2], [1]Roche Inst. of Mol. Biol, Nutley, N.J., 07110, and [2]Univ. of Texas Health Sci. Center, Dallas, Tex. 75235.

Serial undiluted passage of Vesicular Stomatitis Virus (VSV) has been shown to generate defective interfering (DI) particles which contain the same proteins and RNA of the same polarity as the wild-type (wt) virus but are smaller and incapable of self-replication. Replication of DI particles requires co-infection with the wt VSV. The DI particles are capable of synthesizing in vitro only a 46 nucleotide long RNA which is different in sequence from the leader RNA synthesized in vitro by wt VSV. The exception is the DI particle obtained from a heat-resistant strain of VSV (DI-LT) that can transcribe the leader RNA and mRNA species coding for N, NS, M, and G. The former DI particles arise from the 5'-terminal portion of the wt genome RNA and the latter from the 3'-terminal half.

In this report we show that when the purified cores prepared from three different DI particles including DI-LT were pre-incubated with ATP and CTP, reisolated and subsequently incubated in the presence of the β-γ imido analogue of ATP and three other normal ribonucleoside triphosphates, the full-length complementary strands of the DI RNAs were synthesized. Using a similar approach we have also synthesized the full-length complement of the wt genome RNA in vitro. The in vitro plus strand of DI-LT has been annealed to the wt genome RNA and the precise length of deletion was measured by gel electrophoresis of the ribonuclease treated heteroduplex.

754 POST-TRANSCRIPTIONAL DEFECTS AND PEPTIDE STRUCTURAL ALTERATIONS OF A VESICULAR STOMATITIS VIRUS TS MUTANT RESPONSIBLE FOR SPONGIOFORM MYELOPATHY. Joseph V. Hughes and Terry C. Johnson. Division of Biology, Kansas State University, Manhattan, KS. 66506. We recently have been investigating the ts defects of ts G31, a mutant of VSV that can induce a unique central nervous system (CNS) disease in mice characterized by hindlimb paralysis and status spongiosus, quite unlike the rapidly fatal wt-VSV disease. However, the disease may actually be the result of a variant arising in the CNS, since the initial ts G31 cannot be reisolated from the CNS, and reinjection of the nervous system isolate, ts G31BP, results in a similar disease. We have compared a number of properties of the wt-VSV, the initial ts G31, and the CNS isolate ts G31BP: 1) SDS-PAGE revealed the two ts mutant viruses have a larger M protein than the wt-VSV; 2) two dimensional peptide maps revealed a difference between the M protein of the two ts viruses and the wt-VSV M protein; 3) the peptide maps have indicated the 4 other VSV proteins were similar in the three viruses. Ts G31 M protein alterations most likely underlie the ts defective assembly of this virus at the plasma membrane. However, the ts G31BP variant, while maintaining the same defective M peptide map, also appears to be an RNA mutant, not as a result of a defective transcriptase, but rather due to rapid degradation of newly synthesized RNA. Two post-transcriptional defects have been measured in ts G31BP which could be responsible for this degradation: 1) a thermally defective nucleocapsid protein that has an altered interaction with the viral RNA and 2) a reduced ability to synthesize poly-A RNA. Thus, multiple defects in the viral RNA metabolism underlies the ts lesion in ts G31BP; examination of several other CNS isolates taken after ts G31 injection have also revealed a close correlation between RNA ts variants and the unique CNS disease.

755 POST-TRANSLATIONAL MODIFICATIONS IN THE MATURATION OF VESICULAR STOMATITIS VIRUS, David W. Kingsbury and Chung Hsu, St. Jude Children's Res. Hosp., Memphis, TN 38101.

The proteins in vesicular stomatitis virus particles are heterogeneous in electric charge when examined by isoelectric focusing, reflecting post-translational modifications. To assess the functional relevance of these changes, we compared free, nucleocapsid-associated, and membrane-associated viral proteins from infected cells with the proteins in virions. The major nucleocapsid protein, N, exhibited increasing numbers of electronegative variants as it moved through nucleocapsids into virions. In contrast, the transcriptase-associated protein, NS, was more heterogeneous in the free pool than in virions or intracellular nucleocapsids, due to loss of an electronegative species. Lastly, phosphorylated forms of the G, N, and M proteins were detected in virions, but not in infected cells. Thus, a complex series of post-translational modifications accompanies (and may regulate) stages in maturation of the virus.

756 COMPLEMENTATION STUDIES OF NEWCASTLE DISEASE VIRUS (NDV) ts MUTANTS AND PRELIMINARY CHARACTERIZATION OF THE TWO RNA$^-$ GROUPS, A AND E. Mark E. Peeples, Helen R. Kotilainen, Leonida L. Rasenas, James P. Gallagher, and Michael A. Bratt, U. Mass. Medical School, Worcester, MA 01605.

Tsipis and Bratt (J. Virol. 18:848,1976) reported the isolation of a series of ts mutants of NDV strain Australia-Victoria (AV-WT) and a preliminary complementation pattern based on a limited number of crosses among 15 of these mutants. All of the possible complementation crosses of 23 of the ts mutants have now been done. The results confirm the preliminary pattern: i.e., complementation groups A,B,C,D and E, as well as BC which complements with neither group B or C.

The 5 group A mutants are all ts for total RNA synthesis as well as primary transcription. Mutants A2,3,4 and 5 are able to continue synthesizing RNA after a shift to nonpermissive temperature at 3.5 hours PI, or later. Mutant A1 loses its RNA synthetic ability after a similar shift. The single group E mutant (E1) is also ts for total RNA synthesis, but is not ts for primary transcription. Like most of the A group mutants, E1 is able to continue synthesizing RNA after a shift to nonpermissive temperature at 3.5 hours. Velocity sedimentation patterns of the NDV-specific RNA produced at the nonpermissive temperature during primary transcription by E1 do not differ from those produced by AV-WT. The same is true of RNAs produced by A4 and E1 after a shift to the nonpermissive temperature. Thus, we have identified 3 phenotypic classes of mutants within these 2 RNA$^-$ complementation groups: one, ts for primary transcription and for RNA synthesis after a shift up; one, ts for primary transcription but not for RNA synthesis after a shift up; and one, ts for neither.

757 WHAT THE NONCYTOPATHIC (nc) MUTANTS OF NEWCASTLE DISEASE VIRUS (NDV) AND SOME OF THEIR REVERTANTS TELL US ABOUT CYTOPATHOGENICITY AND VIRULENCE, Michael A. Bratt and Charles H. Madansky, University of Mass. Medical School, Worcester, Massachusetts 01605.

The 6 nc mutants isolated by Madansky and Bratt (J. Virol.26:724,1978) as hemadsorption positive spot-formers from the plaque-forming AV strain of NDV and shown to be less cytopathic in single cycles of infection, to produce 10-200% as much virus as the parent, and to be less virulent as indicated by extended mean embryo death times (MDTs) in embryonated hens' eggs have now been shown to: 1) be restricted in viral RNA synthesis; 2) accumulate less L protein intracellularly, and 3) complement with the Group E ts RNA$^-$ mutant, but not the Group A ts RNA$^-$ mutants isolated by Tsipis and Bratt (J. Virol 18:848, 1976). Plaque-forming revertants of 5 of these mutants show at least partial co-reversion of each of these properties, suggesting that RNA synthesizing capacity and cytopathogenicity are causally related.

At least 3 of the nc mutants (those chosen originally as small spot-formers) have a second mutation resulting in either the accumulation of an F protein precursor or an X protein whose origin is still in question. A (small) plaque-forming revertant of the F protein mutant shows no reversion of the F alteration but reversion for RNA synthesis and a reduced MDT. A large spot-forming revertant of the X protein mutant shows no increased RNA synthesis, but loss of the X protein and a reduced MDT. A second step plaque-forming revertant of this spot size revertant shows increased RNA synthesis and a further reduced MDT. The independent reversion of properties relevant to cytopathogenicity and size suggest that these are separate properties and the incremental effect of these mutations on MDTs suggest that each contribute to virulence.

758 Genetic Evidence that Hemagglutinating and Neuraminidase Activities Occupy Different Sites on the HN Glycoprotein of Newcastle Disease Virus. Glenn W. Smith and Lawrence E. Hightower. Microbiology Section, University of Connecticut, Storrs, CT 06268

The HN glycopolypeptide of paramyxoviruses forms projections on the surfaces of infected cells and virions. These projections have both hemagglutinating (HA) and neuraminidase (NA) activities. By analyzing revertants of a temperature-sensitive RNA$^+$ mutant of NDV (isolated by Tsipis and Bratt), we have obtained evidence that these activities occupy different sites on the HN glycoprotein. Revertants were isolated in two steps from a complementation group C mutant which did not form plaques or infectious virus at 42°C (nonpermissive) but which produced infectious virions at 37°C (permissive) with reduced amounts of HN glycopolypeptides and comparable reductions in HA and NA. A revertant was isolated which formed small plaques at 42°C but still carried the defects in HN of the group C mutant. A second revertant was isolated from the cloned small-plaque revertant. It formed large plaques at 42°C like the parental strain AV and its virions contained wild-type amounts of HN glycopolypeptides. Virions of the large-plaque revertant contained wild-type levels of HA but virtually no detectable NA, demonstrating that these two activities can be uncoupled. The lack of NA did not impair the reproduction of the large-plaque revertant in cultured cells nor its virulence in ovo.

759 EXPERIMENTAL RELAPSING MYELITIS IN HAMSTERS ASSOCIATED WITH A VARIANT OF MEASLES VIRUS
Donald R. Carrigan and Kenneth P. Johnson. University of California, San Francisco; San Francisco, California 94143

Chronic relapsing myelitis was induced in hamsters by intracerebral inoculation of measles virus when the animals were less than one day old. Disease appeared from two months to one year after infection and was characterized pathologically by mononuclear cell infiltration, gliosis, and demyelination. Usually the disease was slowly progressive with prominent myoclonus of the hind limbs. In some animals the disease occurred as episodic paresis of the hind limbs with near total recovery between periods of paralysis. Virus could not be demonstrated in lesions by immunofluorescence or isolated by cocultivation of spinal cord with monkey kidney cells. The strain of virus used was distinctive in that it contained high levels of a naturally occurring viral variant, differing from typical measles in several ways. In cultures of monkey kidney cells the variant induces cell rounding and swelling rather than the usual measles syncytia formation. In acutely infected neonate hamster brain the variant grows less readily than does the syncytiagenic form of the virus and is less encephalitogenic. The variant has a decreased buoyant density in potassium tartrate gradients compared to typical measles, yet it expresses the same major structural polypeptides. However, the relative amounts of the two viral envelope glycoproteins are altered in the variant. The variant can be cloned and titered independently of the syncytiagenic type of virus, and is therefore unlikely to be a defective form of typical measles virus. This experimental system will be of value in the study of the immunological and pathogenetic aspects of chronic neurologic diseases associated with unusual or aberrant viral infections.

760 PRODUCTION OF MONOCLONAL ANTIBODIES TO MEASLES VIRUS PROTEINS BY LYMPHOCYTIC HYBRIDOMAS, Michael J. Birrer, Stephen Udem, Barry Bloom, Albert Einstein College of Medicine, Bronx, N.Y. 10461

We have produced stable mouse myeloma antibodies to individual measles virus polypeptides. Immune spleen cells were produced by immunization with either purified measles virus or persistently infected human lymphoblastoid cells. Cell fusion and hybrid isolation was performed by the protocol of Yelton et al. (1).

Detection and characterization of antibody producing hybrids was performed in the following ways: i) Radioimmune assay using I^{125} labeled anti-mouse light chain and virally infected cell extract; ii) neutralization of plaque forming ability of infectious measles virus; and iii) immunoprecipitation followed by SDS acrylamide gel electrophoresis of the disrupted immune complexes.

Hybridomas have been isolated which are reactive against determinants of at least two different measles polypeptides:

(a) Nucleocapsid protein (NC)
(b) Hemaglutinin (P_1 or G).

Data will be presented concerning the use and specificity of these hybridomas including demonstration that the P_4 polypeptide of measles virus is a breakdown product of NC.

(1) Yelton, D.E., B.A. Diamond, S.-P. Kwan, M.D. Scharff. Current Topics in Microbiology, Vol. 81, Lymphocytic Hybridomas, p. 1.

761 SYNTHESIS OF MATRIX AND PHOSPHOPROTEIN IN SSPE VIRUS INFECTIONS, Julie B. Milstien and Adele S. Seifried, Bureau of Biologics, Food & Drug Administration, Bethesda, MD 20205

SSPE virus isolates which remain cell-restricted in culture contain polypeptides equivalent to those of measles virus with the exception of the 70K phosphoprotein (P) and the 38K matrix protein (M) when analyzed on polyacrylamide gels. Electrophoretic analysis shows that these proteins are not detectable in purified virions nor in extracts of cells infected by these SSPE viruses. However, when poly A-containing messenger RNA is selected by dT-cellulose chromatography from the cytoplasms of cells infected by these viruses and used to direct cell-free translation in a reticulocyte lysate system, both M and P polypeptides, in addition to other polypeptides seen in the analogous experiment conducted with measles virus mRNA, are synthesized. Another polypeptide, which appears to be a nonstructural phosphorylated form of M, is synthesized when the reticulocyte lysate translation system is programmed by either measles virus or SSPE virus mRNA's. This polypeptide is also found in cells infected by measles or SSPE viruses, but not in purified virions. These data suggest a model in which deficiency of M and P proteins in SSPE virions may be related to a block in the post-translational conversion of the phosphorylated form of M to that form which is present within purified virions. Though there is no detectable difference in the size of the mRNA for this phosphorylated protein nor of the polypeptide synthesized from it, there may be small base sequence differences in the mRNA resulting in a change in primary and/or secondary structure of the protein which prevents this conversion.

762 INFECTIOUS BRONCHITIS VIRUS-SPECIFIED INTRACELLULAR RNA'S, David F. Stern and S.I.T. Kennedy, University of California San Diego, La Jolla, CA 92093

We have been investigating the synthesis of intracellular RNA species in cells infected with the avian coronavirus Infectious Bronchitis Virus (IBV). Chicken embryo kidney cells were infected with IBV in the presence of actinomycin D and labeled with ^{32}P-orthophosphate. Nucleic acids were extracted, denatured, and analyzed on agarose gels. Six major RNA's were seen which did not appear in mock-infected extracts. These RNA's are all single stranded and consist of the intracellular genome (molecular weight 8.1 X 10^6) and 5 other single stranded RNA's with apparent molecular weights of 0.79 X 10^6, 0.91 X 10^6, 1.27 X 10^6, 1.45 X 10^6, and 2.6 X 10^6. All of the RNA's are polyadenylated and therefore are likely to be viral mRNA's.

The four smallest RNA's and the genome were analyzed by ribonuclease T1 fingerprinting. The results indicate that the RNA's are derived from the genome and are therefore subgenomic and that the RNA's constitute a nested set such that the sequences of each RNA are contained within those of the larger RNA's, with each larger RNA gaining additional sequences congruent with its increase in size. We are currently determining a 5' to 3' oligonucleotide spot order for the genome to enable mapping the subgenomic RNA's with respect to one another and to the genome. This project is one part of our overall study of the IBV replication strategy.

763 GENETIC ANALYSIS OF THE RESISTANCE TO MURINE HEPATITIS VIRUS, STRAIN JHM, INDUCED ENCEPHALOMYELITIS, Stephen Stohlman and Jeffrey Frelinger, University of Southern California, School of Medicine, Los Angeles, CA 90033

The JHM strain of murine hepatitis virus (MHV), a coronavirus, causes an acute encephalomyelitis with both acute and chronic demyelination. Analysis of host resistance to JHM virus showed that: 1) only SJL of the 15 strains of mice tested resisted intracranial challenge with 1000 SMB LD_{50}, 2) resistance is not solely H-2 associated since two other H-2^s strains (B10.S and A.SW) were completely susceptible, 3) and resistance is mediated by two genes, Rhv-1 and Rhv-2. Backcrosses and F_2 analysis suggested that Rhv-1^R was dominant and that Rhv-2^r was recessive. Crosses designed to discover strains with the genotype Rhv-1^s, Rhv-2^r indicate that Balb/c carries such a genotype since (SJL X Balb/c) F_1 are resistant while all other F_1s tested have been susceptible.

A single gene has been described which controls resistance to MHV induced hepatitis. The C3H/Bang strain is resistant and the PRI is susceptible. A congenic line, C3H.SS, carries the susceptible allele. To test whether this gene might be identical to either Rhv-1 or Rhv-2 these mice were challenged intracranially with JHM virus. C3H.SS is resistant to JHM, while C3H/Bang is susceptible. This indicates that either Rhv-1 or Rhv-2 also governs resistance to MHV induced hepatitis.

764 Neurovirulence and persistency of mouse hepatitis viruses in rats
H. Wege, M. Koga, S. Siddell, J. Stephenson and V. ter Meulen
Institute for Virology, University of Würzburg, W.-Germany

The murine coronavirus JHM induces different types of central nervous diseases in weanling rats after intracerebral injection ranging from an acute panencephalitis to a late demyelinating encephalomyelitis. The lesions in the acute panencephalitis consist of widespread necrosis in all parts of the central nervous system accompanied by acute inflammations and destruction of both neurons and oligodendroglia. The subacute demyelinating encephalomyelitis is characterized by demyelination in the absence of acute inflammations and preservation of both axons and neurons. The late demyelinating encephalomyelitis develops after an incubation period of 2 - 8 months and shows both demyelination and remyelination of axons. Infectious virus can be isolated in all three disease patterns even after an incubation period of months. The occurrence of the different types of diseases depends on the properties of the virus clones used as inoculum.

Other murine coronaviruses (A59, MHV2 and MHV3) induce CNS infections in rats without demyelination. The relationship of these strains to JHM was investigated by antigenic analyses and oligonucleotide mapping of their genomic RNA.

765 THE EXPRESSION OF A CORONAVIRUS GENOME INVOLVES MORE THAN ONE SUB-GENOMIC mRNA, Stuart G. Siddell, H. Wege, A. Barthel and V. ter Meulen, Institut für Virologie, Universität Würzburg, 8700 Würzburg,W.-Germany

The structure of several coronaviruses has recently been described but very little is known of their replication. Cells infected with the murine coronavirus strain JHM shut-off host cell protein synthesis and synthesize a number of virus-specific proteins including two structural proteins, p60 and p23. The poly A RNAs encoding p60 and p23 can be isolated from the cytoplasm of infected cells and translated in both reticulocyte and L-cell cell-free systems to give authentic products which can be identified by specific immunoprecipitation and tryptic peptide fingerprinting. The RNA encoding p60 sediments in sucrose-formamide gradients at 17S and that encoding p23 is clearly seperable and sediments at 19S. Both RNAs can be specifically released from infected cell polysomes and are therefore physiological mRNAs and they both constitute a large proportion of the viral RNA synthesized late in infection. Our interpretation of this data is that these RNAs represent two subgenomic coronavirus JHM mRNAs each of which encodes a different structural protein of the virus and if correct shows that the replication strategy of coronaviruses contrasts with other well-characterized non-transforming positive-stranded RNA animal viruses.

766 POLYPEPTIDE HOMOLOGY BETWEEN TROPISM-CONTRASTING STRAINS OF MOUSE HEPATITIS VIRUS, Stephen B. Cheley and Robert Anderson, *Dept. of Microbiology & Immunology, University* of Western Ontario, London, Canada

Studies in our laboratory provide biochemical evidence for the derivation of a highly neurotropic (JHM) strain from the more prevalent and primarily hepatotropic (MHV3) strain of mouse hepatitis virus. Both strains code for three major primary gene products: a large glycoprotein, p'180', a basic, phosphorylated protein, p'56' and a low molecular weight protein, p'22'. Proteins p'56' and p'22' give rise to product polypeptides p'50' and p'24' respectively through post-translational modification mechanisms. All polypeptides of JHM have only slightly lower apparent molecular weights in SDS PAGE than the corresponding ones of MHV3. Comparison of polypeptides from MHV3 and JHM by proteolytic peptide mapping shows a high degree of structural relatedness between the two strains. Discrete peptide differences are, however, detected. Since virus-specified tissue tropism is commonly determined by structural characteristics of viral polypeptides, it is suspected that the observed peptide differences between MHV3 and JHM may contribute to the biological differences exhibited by the two strains.

767 REPLICATION OF MEASLES VIRUS IN THE PRESENCE OF TUNICAMYCIN. Kathryn C. Stallcup and Bernard N. Fields, Harvard Medical School, Boston, MA 02115

Tunicamycin (TM) was used to inhibit the addition of sugars to the glycoproteins of measles virus in order to study the role of carbohydrates in the strucutre and function of these proteins. 1.0 µg/ml of TM, administered 2 hrs post infection to cell cultures infected with virus, completely stopped the production of infectious progeny virus. This inhibition occurred at 33°C and 37°C and affected both the Edmonston and Joy wild type measles strains. The release of material with the density of virions was inhibited 85-90%. Synthesis of the nonglycosylated viral proteins (L, P, NP, M) appeared to be normal in the presence of the drug, while changes were observed in the synthesis of H, the large viral glycoprotein. Particles released from TM-treated cultures lacked the normal H protein, but did contain a polypeptide, termed p90, which migrated more slowly than H on SDS-PAGE. p90 was also found in particles released from cultures infected in the presence of 2-deoxy-D-glucose. Partial protease digests indicated that the p90 polypeptide might be related to H. The anomalously slow migration of p90 suggests that there may be proteolytic processing of the H protein.

768 Antigenic Variants of Visna Virus Have Genetic Changes Clustered in the 3' Terminal Region of the Genome. J.E. Clements*, N.D'Antonio*, O. Narayan*, F.S. Pedersen[O] W.S. Haseltine[O]. *Johns Hopkins School of Medicine, Baltimore, Maryland 21205. [O]Harvard Medical School, Boston, Massachusetts 02115.

Antigenic variants of visna virus have been compared using the genomic RNA and analyzing the large RNase T_1-resistant oligonucleotides. Mutants isolated from a persistently infected sheep contained a small number of changes in their oligonucleotide patterns when compared with parental virus. To determine whether the changes in the nucleotide structure were clustered in one region of the genome, the oligonucleotides of the parental and a mutant RNA were ordered along the genome with respect to the 3' polyadenylated end. All but one difference between the parental strain and the antigenic mutant used for mapping were located within 2 kilobases from the 3' terminus. The electrophoretic mobilities of some of the oligonucleotides which differed from the parental to the mutant suggest that they might be derived by simple mutation. To determine whether this was how the mutants arose the complete nucleotide sequence of several oligonucleotides were determined. These analyses showed that a number of the oligonucleotides which were different between the parental and mutant could be accounted for by single base changes. Thus, based on these studies we propose that multiple mutational events in the region of the genome which codes for the viral antigen that elicits neutralizing antibody permit the virus to escape the immune response and may, in addition, be responsible for the slow progressive nature of the disease.

Segmented RNA Viruses

769 THE INFLUENZA VIRUS HAEMAGGLUTININ GENE : CLONING AND DNA SEQUENCE OF DOUBLE STRANDED COPIES OF THE HA GENES FROM H_2 AND H_3 PANDEMIC VIRUSES. Mary-Jane Gething, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England.

Approximately 70% of the amino acid sequence of the HA from Influenza strain A/Japan/307/57 (pandemic group H_2N_1) has been determined in this laboratory using protein chemistry techniques (Waterfield et al., Brit. Med. Bull. 35, 57 (1979)). However analysis of certain regions of the molecule, including the hydrophobic membrane associated peptides, has proved difficult. Thus cDNA copies of HA virion RNA have been cloned and sequenced in order to complete the determination of the primary structure of the molecule. The HAs chosen for analysis were from Influenza strains A/Japan and A/X-31 (pandemic group H_3N_1). Sequence data for X-31 HA is now required for interpretation of X-ray crystallographic data by D. Wiley, Harvard, in order to determine the 3-dimensional structure of the protein. Virion RNAs from these strains have been purified and double stranded cDNA copies have been synthesized using reverse transcriptase and DNA polymerase I. The cDNA copies were inserted into plasmid pAT153 at the Pst I restriction site and cloned and propagated in *E.coli* X1776. Recombinant plasmids containing DNA which hybridized to a purified HA probe have been amplified, purified and analysed using restriction endonuclease digestion. One such plasmid contained an insert comprising approx. 95% of the nucleotide sequence coding for Japan HA. Another contained approx. 50% of the X-31 gene.Restriction fragments from the distal ends of these inserts have been utilized to prime synthesis of cDNA copies and to clone the missing portions of the HA genes. Complete DNA sequence analysis of these inserts allows comparison of the primary structures of HA molecules from two different pandemic strains.

770 INFLUENZA VIRUS PROTEINS INVOLVED IN VIRAL RNA SYNTHESIS; Adrian J. Wolstenholme and Thomas Barrett, Department of Pathology, University of Cambridge, Cambridge, U.K.

The synthesis of virus-specific RNA and proteins was examined in primary chick embryo fibroblast cells infected with temperature-sensitive mutants of fowl plague virus. The defective proteins in these mutants had been found previously to be the P_1, P_2, P_3, NP and NS proteins (Almond *et al.*, Virology 92, 416-427 (1979)). Mutants in the P_2, P_3, and NP proteins were found to be defective in the synthesis of all viral proteins and RNA at the non-permissive temperature (40.5°), whereas early protein and RNA synthesis continued normally in cells infected with mutants in the P_1 and NS proteins at 40.5°. None of the mutants was capable of synthesising new negative-stranded genome RNA at the non-permissive temperature, even when allowed to synthesise non-polyadenylated template cRNA at the permissive temperature. In addition, these mutants failed to switch to the synthesis of the late virus proteins at 40.5°, suggesting a link between the switch to late protein synthesis and the start of new vRNA synthesis in fowl plague virus-infected cells.

771 THE STRUCTURE AND EXPRESSION OF INFLUENZA GENES CLONED IN BACTERIAL PLASMIDS,

Norman H. Carey, Alan G. Porter, William C.A. Tacon, Geoffrey Threlfall and Spencer Emtage, Searle Research Laboratories, England.

Gene 4, the haemagglutinin gene, of both human (A/Victoria/3/75) and chicken influenza viruses (FPV) and gene 8 of FPV (coding for NS1 and NS2) have been reversed transcribed into double stranded DNA copies after in vitro polyadenylation of the viral RNA. The transcripts were combined with pBR322, cloned in E. coli and complete sequence analyses obtained. The nucleotide sequences predict the complete amino acid sequence of the mature HA protein and its precursor and, in the case of gene 8, indicate the mechanism by which NS1 and NS2 are produced from this RNA segment.

The HA genes have been transferred to plasmids derived from the trp promoter region of the E. coli genome and designed to achieve controlled expression of sequences inserted at a specific site. Such plasmids direct the synthesis of a protein that reacts specifically with antiserum to FPV-HA. The production, characterisation and relationship of this protein to authentic HA will be discussed.

772 PERSISTENT INFECTIONS OF BUNYAVIRUSES IN AEDES ALBOPICTUS. Robert Z. Florkiewicz and Martinez J. Hewlett, University of Arizona, Tucson, Arizona 85721.

A continuous mosquito cell line, Aedes albopictus, has been infected with the Bunyaviruses Uukuneimi or Inkoo resulting in the establishment of a "persistently" infected cell state. A one-step growth curve of Inkoo virus in the Aedes albopictus cell line gives a peak virus titer in the medium of 2×10^{10} PFU per ml at thirty hours post-infection. However, after infected cells are passed eight times the titer of virus released into the medium is 1×10^{4} PFU per ml. In contrast the one step growth curve of Uukuneimi in Aedes albopictus yields 1×10^{6} PFU per ml at thirty hours post-infection. After eight passages of these infected cells no plaque forming units can be detected in the medium. The intracellular viral RNA and viral RNA isolated from extracellular virus pelleted from the medium from the persistently infected cell lines has been compared with RNA of virus isolated from infected BHK cells as well as from twenty-four post-infection Aedes albopictus cells. Analysis of these RNA's indicated a preferential loss of the M and probably the S genome segments with the concomient appearance of a small (less than 4S) class of molecules. The profile obtained with rival RNA from both Uukuneimi and Inkoo persistently infected cells is nearly identical. The coinfection of wild type virus with virus from persistently infected Aedes albopictus cells interfers with the pattern of wild type growth in BHK cells. Interference is most striking at 28° and is essentially undetectable at 37°. This suggests a temperature sensitive phenomina superimposed over a defective interfering activity. We propose that this system be used as a model for both the generation of the persistently infected cell state as well as for the horizontal transmission of this and similar viruses from invertibrate to vertibrate hosts.

773 GENETIC VARIATION DURING PERSISTENT REOVIRUS INFECTION, Rafi Ahmed and B.N. Fields, Department of Microbiology, Harvard Medical School, Boston, MA 02115

A persistent reovirus infection was established by infecting L cells with a serially passaged stock of temperature-sensitive (ts) mutant C(447) containing greater than 90% defective interfering (DI) particles. The nature of the virus produced in this carrier culture was examined over a period of 1 1/2 years. We have found that (1) the phenotype of the virus changes from ts to ts^{+} (wild type) within a few weeks of the initial infection (2) some of the ts^{+} clones contain extragenically suppressed ts lesions (3) ts mutants in recombination groups B, G and a new group, designated H, were rescued from the suppressed clones (4) deletion mutants (DI particles) lacking genomic dsRNA segments L1, L3 and M1 are present in the carrier culture. These results show that reovirus undergoes extensive mutation during persistent infection.

Since persistently infected cells contain both infectious virus and DI particles, there should be ample opportunity for genetic interaction between them. We have found that reovirus DI particles can reassort genes with infectious virus and it is possible to rescue ts lesions from the DIs. Thus, reovirus DI particles in addition to lacking certain dsRNA segments also contain mutations in other genes. It is possible that DI particles provide a source of mutant genes and that the genetic variability of virus isolated from the persistently infected cells is due to recombination between DI particles and infectious virus.

774 TERMINAL SEQUENCE HOMOLOGY IN THE ds RNAs, AND THEIR PLUS-STRANDED TRANSCRIPTS, OF THE THREE SEROTYPES OF REOVIRUS. Joseph K.-K. Li, Jack D. Keene, Patricia Scheible and Wolfgang K. Joklik, Dept. of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.

Type specificity, neurovirulence and hemagglutination are all specified by reovirus polypeptide σ1 which is encoded by genomic RNA segment S_1. In order to investigate the molecular basis of the biological differences and the genetic relatedness among reovirus serotypes 1, 2 and 3, we have determined the nucleotide sequences of portions of their respective S_1 RNAs. The 3'-termini of S_1 RNA and of its plus-stranded transcript (synthesized by cores) were ligated with ^{32}pCp and sequenced (chemical sequencing and homochromatography). The 3'-ends of the plus strands of the S_1 segments of the three serotypes, as well as of their transcripts, possess extensive terminal homology which includes five common terminal residues (pUpCpApUpC-OH), a G-rich region and six further homologous residues. The 3'-ends of the minus strands of the S_1 segments of the three serotypes share six common terminal residues (pApApUpApGpC-OH) and this sequence is also present at the 3'-end of the minus strand of the S_2 segment of serotype 3; further it is complementary to the sequence m^7GpppG^{m}pCpUpAp known to be present at the 5'-ends of six species of reovirus serotype 3 plus-stranded RNA molecules. The 3'-ends of the plus strands of the S_1 and S_2 segments of serotype 3 are identical for the first six residues but then diverge completely, unlike the sequences at the 3'-ends of the plus-strands of the S_1 segments of serotypes 1, 2 and 3, which resemble each other closely for about the first 20 residues.

775 INFECTION OF MOUSE EMBRYOS AND UNDIFFERENTIATED TERATOCARCINOMAS WITH REOVIRUSES, Jan Abramczuk, Andrzej Vorbrodt, Bernard N. Fields and Hilary Koprowski. The Wistar Institute, Philadelphia, PA 19104 and Harvard Medical School, Boston, MA 02115.

Mouse one- and two-cell embryos were destroyed at the cleavage stage when they were cultured in the presence of reovirus type 1 or type 3. The lethal concentration of reovirus 1 was one hundred times lower than that of reovirus 3 (3×10^7 particles/ml and 3×10^9 particles/ml, respectively). At lower concentrations of virus particles, the destruction of embryos was delayed and occurred after blastocyst formation.

Infection of blastocyts and inner cell masses isolated from blastocysts also resulted in their destruction, the lethal concentration of reovirus 1 being again much lower than that of reovirus 3. Electron microscope observation showed the presence of microtubules in the viral factories in embryos infected with reovirus 1 but not in embryos infected with reovirus 3.

Mouse teratocarcinoma cells (F9 and PCC4) were infected with reovirus 1 or 3 at multiplicity of 2.5×10^3 particles/cell. Indirect immunofluorescence staining with anti-reovirus sera revealed that, depending on the time after infection, 20-100% of cells contained viral inclusions.

Our results demonstrate the susceptibility of undifferentiated embryonal cells to reovirus infection and the existing difference in the virulence between both types of reoviruses during early stages of mouse development.

776 ASSIGNMENT OF REOVIRUS mRNA RIBOSOME BINDING SITES TO VIRION GENOME SEGMENTS BY NUCLEOTIDE SEQUENCE ANALYSIS, Edward Darzynkiewicz and Aaron J. Shatkin, Roche Institute of Molecular Biology, Nutley, NJ 07110

All ten reovirus genome RNA segments were radiolabeled at their 3'-termini by incubation with RNA ligase and ^{32}pCp. The extent of radiolabeling was similar for each of the double-stranded RNAs in the genome segment mixture. Radioactivity was equally distributed between the separated plus and minus strands indicating that the 5'-cap in plus strands did not block 3'-end-labeling of minus strands. The 3'-termini of the four S and three M segments included common but different sequences in plus and minus strands. By comparing the minus strand 3'-sequences with 5'-sequences of reovirus mRNAs, small-size genome segments S2, S3 and S4 were correlated with the previously reported initiation fragments s46, s45 and s54 derived from small class mRNAs (M. Kozak, Nature 269:390, 1977). Medium-size genome segments M1, M2 and M3 similarly were correlated with fragments m30, m52 and m44, respectively. The N-terminal amino acid sequences deduced from the mRNA nucleotide sequences can now be assigned to the nascent chains of particular reovirus proteins as follows: viral core polypeptide σ2 = Met-Ala-Arg-Ala-Ala-Phe-Leu-Phe; non-structural protein σNS = Met-Ala-Ser-Ser-Leu; virion major outer shell polypeptide σ3 = Met-Glu-Val-Cys-Leu-Pro-Asn, coded for by genome segments S2, S3 and S4, respectively (M.A. McCrae & W.K. Joklik, Virology 89:578, 1978; T.A. Mustoe et al., Virology 89: 594, 1978); and minor virion polypeptide μ2 = Met-Ala-Tyr-Ileu-Ala; cleaved virion polypeptide μ1 = Met-Gly-Asn-Ala; and non-structural protein μNS = Met-Ala-Ser-Phe-Lys-Gly-Phe-Ser, coded for by segments M1, M2 and M3, respectively.

777 SEPARATION OF FULL LENGTH TRANSCRIPTS AND GENOME RNA PLUS AND MINUS STRANDS OF CYTOPLASMIC POLYHEDROSIS VIRUS OF BOMBYX MORI, Robert E. Smith and Yasuhiro Furuichi, Roche Institute of Molecular Biology, Nutley, NJ 07110

The transcription of Cytoplasmic Polyhedrosis Virus (CPV) of Bombyx mori, a ten-segmented dsRNA virus, was investigated. Efficient in vitro synthesis of CPV mRNA was facilitated by the presence of sodium acetate, high concentrations of ribonucleoside triphosphates and proteinase K. Under optimal conditions, transcription was maintained for more than 24 hr, resulting in large quantities of full-size mRNAs which were active in cell-free translation. A mixture of CPV mRNAs consisting of ten RNA species was resolved into nine discrete bands by agarose gel electrophoresis with 7 M urea. The genome RNA segments coding for each mRNA were identified by hybridizing the separated, ^{32}P-labeled mRNAs to a mixture of genome RNAs. Each mRNA hybridized uniquely with its corresponding genome RNA segment in order of decreasing size. Each of the genome segments appear to be transcribed at the same rate producing equal quantities by weight but different molar amounts of each of the separated mRNA species. These results imply that each of the genome segments is transcribed repeatedly by an equal number of template-associated RNA polymerases. The genome RNAs of CPV and reovirus, labeled at the 3'-termini with ^{32}pCp by RNA ligase, were separated by agarose gel electrophoresis into the plus and minus strands. The RNA strands of plus polarity were found to migrate faster in the gel than those of minus polarity for all CPV dsRNA genome segments, whereas the opposite was seen for most of the reovirus genome segments. These findings should provide a simple method for preparation of purified single-stranded RNAs of plus or minus polarity from each segmented dsRNA genome for sequence analysis.

778 A POSSIBLE INVOLVEMENT OF mRNA-METHYLTRANSFERASES IN THE INITIATION OF RNA SYNTHESIS BY CYTOPLASMIC POLYHEDROSIS VIRUS OF BOMBYX MORI, Yasuhiro Furuichi[1], Alice M. Wertheimer[1], Yih Shiong Wu[2] and Ronald T. Borchardt[2], [1]Roche Institute of Molecular Biology, Nutley, NJ 07110 and [2]University of Kansas, Lawrence, KS 66045

Cytoplasmic polyhedrosis virus (CPV) of the silkworm contains a ten-segment dsRNA genome, a virus-associated RNA polymerase and enzymes required for the formation of a 5'-cap in the mRNAs. Messenger RNA synthesis in vitro by CPV is stimulated by the presence of the methyl donor S-adenosylmethionine (AdoMet) (Furuichi, Nuc. Acids Res. 1:801, 1974). The stimulatory effect by AdoMet is probably at the initiation step of RNA synthesis. In an effort to determine the important structural features of the AdoMet molecule required for the stimulation, analogs of AdoMet have been tested for their ability to promote RNA synthesis and to inhibit AdoMet-stimulated methylation. Several potent inhibitors of cellular and viral methyltransferases, e.g., S-adenosylhomocysteine (AdoHcy), 3-deaza AdoMet, 7-deaza AdoMet, 7-deaza AdoHcy, and the antibiotic Sinefungin, inhibit methylation of CPV mRNA, but stimulate mRNA synthesis as effectively as AdoMet. In contrast, most of the analogs which fail to inhibit CPV methyltransferase also fail to stimulate CPV mRNA synthesis. Structural features of AdoMet needed for efficient mRNA synthesis apparently coincide with those recognized by CPV methyltransferase. These results suggest that binding of AdoMet to methyltransferase (or some other AdoMet-recognizing protein) in CPV transcriptional complexes activates the RNA polymerase through an AdoMet-mediated allosteric conformational change resulting in the mRNA synthesis and the formation of the methylated cap structure.

779 POSSIBLE MECHANISM OF ROTAVIRUS PERSISTANCE, Vikram Misra and Lorne A. Babiuk, Dept. Vet. Microbiology, WCVM, Univ. of Saskatchewan, Saskatoon, Sask., Canada.

Rotaviruses have been implicated as one of the etiological agents responsible for neonatal diarrhea in man and other animals. The disease in calves is seasonal although the mechanism by which the virus persists from season to season is not known. Most isolates of bovine rotavirus are cytolytic for BSC-1 cells. Infection causes breakdown of host DNA and cessation of host macromolecular synthesis followed by death of the cell and release of infectious virus. One isolate (2352) however, appears to be deficient in its ability to shut-down host synthesis and causes a persistant infection in BSC-1 cells (RP-BSC-1). Viral persistance does not adversely affect these cells which grow at a rate comparable to that of uninfected cells. In addition, RP-BSC-1 cells continuously produce Rotavirus and are immune to super-infection with related viruses. To further characterize virus-cell interactions during persistant infections, viral expression was examined in synchronized RP-BSC-1 cells. Viral expression was limited to mid and late G1 when 80% of cells exhibited viral antigens detectable by immunofluorescence and PAGE. Entry into S-phase caused a degradation of viral proteins and a sharp decline in viral expression. Degradation was probably brought about by factors synthesized in S-phase as exponentially growing BSC-1 cells yielded only 'complete' virus particles. The results suggest that some strains of rotavirus are unable to shut-down host macromolecular synthesis and therefore can only replicate in certain permissive phases of the cell cycle. As the infected cell traverses from 'G1' into 'S' viral replication and expression ceases, only to resume when the cells re-enter a permissive phase. Experiments to determine the implications of these observations to persistance in vivo are in progress.

780 POLYADENYLYLATION OF REOVIRUS PROTEINS: STRUCTURE OF THE BOUND RNA. Carol A. Carter Beth Y. Lin, and Marie Metlay. SUNY at Stony Brook, New York, N.Y. 11794

The reovirus oligoadenylates exist in two states within the virion: free and bound to viral proteins. The covalently bound RNA exists in two types of linkages: Oligo(A), n=1-6, is bound through its 3'-terminus, and oligo (ADP-ribose), $n \geq 1.5$, is bound through the ribose moiety to protein. The structurally related outer shell proteins, μ_1, μ_{1C}, and viii are modified to the greatest extent, both in virions and in infected cells. Purified virions contain enzymatic activities which catalyze the formation of both types of modification. Moreover, *in vitro*, modification of both viral proteins and ds RNA occurs. The results suggest that reoviruses are equipped with novel forms of phosphorylated proteins. If adenylylation and ADP-ribosylation, facilitate RNA-protein interactions, they may play important roles in selection of the proper number and class of genome segments during maturation.

Immunogenetics of Animal Viruses

781 PROPERTIES OF A REPLICATION-DEFECTIVE MURINE LEUKEMIA VIRUS PRODUCED BY CULTURED AKR LEUKEMIA CELLS, Alan Rein, Brenda I. Gerwin, Sandra K. Ruscetti, Douglas R. Lowy, Robert H. Bassin, Eleni Athan, and Beryl M. Benjers, National Cancer Institute, Bethesda, Md. 20205

As recently reported by Nowinski et al., cell lines derived from AKR leukemia tissues do not produce non-defective ecotropic MuLV (Virology 81:363). We have recently shown that one of these lines, AKRSL2, releases a replication-defective MuLV as well as an MCF-like virus (Rein et al., Nature, in press). In order to characterize the former MuLV in molecular terms, we have looked for viral products in "non-producer" clones containing the defective MuLV. These experiments indicate that the defective MuLV directs the synthesis of an ecotropic env protein. However, RIAs and immune precipitation tests show that these non-producer cells do not contain several (and possibly all) gag proteins at detectable levels. Preliminary restriction mapping data suggest that the defective MuLV may contain a deletion in the 5' half of the genome, and are thus fully consistent with the apparent absence of gag proteins.

782 ULTRASTRUCTURE OF VIRIONS PRODUCED BY TWO NEW HUMAN EMBRYONAL CARCINOMA CELL LINES, David L. Bronson and Elwin E. Fraley, University of Minnesota Medical School, Minneapolis, Mn. 55455

Particles with the morphology of retrovirions were previously observed at low frequency in two human embryonal carcinoma (EC) cell lines. Virus production was stimulated by incubating cells with 5-iodo-2'-deoxyuridine (IUdR) and dexamethasone (DXM) (J. Natl. Cancer Inst. 60: 1305, 1978; vol. 63:337, 1979). Two new EC cell lines were established from a primary tumor (2102E-P) and from a retroperitoneal lymph node metastasis (2102E-R) from a patient with teratocarcinoma. After treatment of the cells with IUdR and DXM, virions were also detected in 5-10% of cell sections from each of these new lines. The virus buds from the plasma membrane and extracellular particles have a diameter of 100-120 nm, an electron-lucent core of 50 nm, and a smooth envelope. In contrast to type C virions, the particles lack an electron-lucent space between the nucleocapsid and the envelope. However, a few (5% or less) particles observed in both 2102E lines also have surface projections on the viral envelope. These spiked particles have not been detected in any of our other human tumor cell lines. Thus, these two lines, established from different tumor specimens from the same patient, produce either (a) two different types of virions; (b) one virion which occasionally acquires the spiked envelope of another, as yet undetected, virus; or (c) one virion that also has an aberrant form. These EC cells also undergo alterations in cellular morphology, which is largely dependent upon culture conditions and is suggestive of in vitro differentiation. Therefore, the low frequency of virus production might result from a relationship between virus production and the specific stage of each cell in the differentiation program.

783 RECOMBINATION AND PARALYSIS-INDUCTION BY WILD MOUSE AMPHOTROPIC MuLV, Bijay K. Pal, Suraiya Rasheed and Murray B. Gardner, USC School of Medicine, Los Angeles, CA 90033

Serial in vivo passage in newborn NIH Swiss mice of cloned amphotropic MuLV of wild mouse origin generally resulted in a moderate incidence (~25%) of non-T-cell splenic lymphomas within ten months. Occasionally, however, lower limb paralysis, typical of that neurologic disease induced by cloned wild mouse ecotropic virus, developed in the amphotropic virus inoculated mice after a prolonged latent period (>10 months). In three such instances the virus recovered from the serum, spleen and CNS of the paralyzed mice was a mixture of amphotropic and ecotropic virus with the latter producing large XC plaques. Repeated cloning of the original amphotropic virus inoculua did not reveal any contaminating ecotropic virus. To determine whether recombination with endogenous NIH Swiss proviral sequences resulted in formation of these large plaque-forming ecotropic viruses, two-dimensional tryptic peptide mapping was done on the envelope gp70 and gag gene pp12 of these newly derived ecotropic viruses. The gp70 and pp12 maps were different from those of the corresponding proteins of parental amphotropic virus and from ecotropic viruses of wild mouse origin. Our results suggest that both env and gag regions of the amphotropic virus recombined non-randomly with NIH Swiss cellular genes. However, the newly derived recombinant ecotropic viruses did not induce paralysis on further in vivo passage although they were lymphomagenic. Thus, the paralysis-linked amphotropic virus recombinational event may be an interesting epiphenomenon rather than a critical step in the causation of paralysis. (Supported by NCI contract NO1-CP8-1032, an ALSSOA grant, and NIH grant 1-R01-NS14891-01A1.)

784 CHARACTERIZATION OF AN UNIQUE DEFECTIVE C-TYPE VIRUS ASSOCIATED WITH A MoLV INDUCED SPLENIC T-CELL LYMPHOMA CELL LINE, Ivan Horak, John C. Lee, Luis Enjuanes and James N. Ihle, NCI, Frederick Cancer Research Center, Frederick, MD 21701.

Moloney leukemia virus (MoLV) induces lymphomas 3-5 months after inoculation of BALB/c mice. The resulting tumors however are heterogeneous and have been shown to involve immature thymic T cells or a more mature population of splenic T cells (*). Since the latter type of lymphoma is unusual we have established a cell line (5F4) from such a tumor, and have characterized the virus associated with it. The 5F4 cells produce viral particles of C-type morphology which sediment at 1.16 g/cm^3 in isopynic sucrose gradients. No infectious particles however could be detected by infectious center assays on RL-12 thymocytes, FG-10 cells or mink-lung cells indicating that the 5F4 virus was defective for replication. To determine the nature of the defect, virus was examined by competition radioimmunoassays (RIA) for various viral gene products and by enzyme activity for reverse transcriptase. By RIA both p10 and p30 were found at normal levels however, no MoLV p12 or gp71 was detectable although normal levels of AKR MuLV p12 or gp71 were found. These results suggest that although, the 5F4 lymphoma was induced by MoLV the tumor cells were only replicating virus particles serologically related to the endogeneous ecotropic virus. The reverse transcriptase activity of the purified viral particles was only 2-5% with an exogeneous template and 15% with endogeneous template when compared to control viruses, suggesting that the defectiveness of the virus is due to deletion or altered expression of the reverse transcriptase. Virus rescue experiments are currently in progress to determine the possible transforming activity of this defective virus.

*Pepersack, L., Lee, J.C., McEwan, R., and Ihle, J.N., J. Immunol. (In press).

785 Akvr1, A DOMINANT MuLV RESTRICTION GENE IS POLYMORPHIC IN LEUKEMIA-PRONE WILD MICE, Murray B. Gardner, USC School of Medicine, Los Angeles, California 90033

A new restriction gene (Akvr-1, for AKR virus restriction) is polymorphic for two alleles, Akvr-1^R (restrictive) and Akvr-1^r (susceptible) in a leukemia-prone feral mouse (Mus musculus domesticus) population located at a squab farm near Lake Casitas (LC) in southern California. Akvr-1^R is a dominant allele that exhibits 100% penetrance in prevention of both viremia of AKR endogenous retrovirus and of virus mediated lymphoma in LC (Akvr-1^{RR}) X AKR F_1 hybrids. The restriction phenotype segregates as a single Mendelian locus in F2s and in backcrosses to AKR mice. The observed genotype frequencies in LC mice do not vary significantly from expectations of the Hardy-Weinberg equilibrium. Akvr-1^R likewise is effective in restriction of NB-tropic Moloney MuLV-induced viremia and NB-tropic Friend virus-induced splenomegaly, but fails to restrict expression or pathogenesis of LC derived amphotropic retrovirus. Pleiotropic restriction of AKR, Friend and Moloney ecotropic viruses, but not of amphotropic virus, suggests that the viral targets of Akvr-1 in the three ecotropic viruses are similar to each other and distinct from the target in the LC amphotropic virus. The locus appears phenotypically distinct from Fv-1, Fv-2 and Fv-3 genes but cannot at this time be separated from the Fv-4 locus described in Japanese mice (Mus molossinus).

786 HIGH SUSCEPTIBILITY TO SARCOMA VIRUS TRANSFORMATION OF SKIN FIBROBLASTS FROM GENETICALLY CANCER-PRONE INDIVIDUALS, Suraiya Rasheed and Murray B. Gardner, USC School of Medicine, Los Angeles, CA 90033

Fibroblasts from certain genetically cancer-prone persons were earlier shown to be unusually susceptible to transformation by RNA or DNA tumor viruses. Later, growth abnormalities and high susceptibility to transformation by Kirsten sarcoma virus (KiMSV/MuLV) of skin fibroblasts from patients with hereditary adenomatosis of the colon and rectum (ACR) and its Gardner's syndrome (GS) variant were reported. We have confirmed these findings and show, in addition, that these cells are also susceptible to transformation by KiMSV with a baboon retrovirus helper (KiMSV/BaEV) or by feline sarcoma virus (FeSV). Both KiMSV/MuLV and KiMSV/BaEV transformed the ACR and GS cells with 300-1000-fold greater efficiency than normal, age-matched, control cells. It is estimated that $>10^3$ focus forming particles are required to induce few foci in normal cells whereas <100 particles are sufficient to transform ACR and GS cells. The relative focus inducing titers of KiMSV (BaEV) were higher than KiMSV (MuLV) in the cells tested. However, the differential transformation efficiency of these viruses in ACR and GS as compared to control cells was not due to differences in the extent of virus infection or replication. The increased transformability of ACR and GS cells thus presumably operates at the host genome level although permanent lines of completely transformed cells have not yet been established. This assay is highly reproducible and shows promise for detection of young ACR or GS gene-bearing "normal" individuals before development of colon polyps and other tumors.

787 GENETIC CONTROL OF MOLONEY LEUKEMIA VIRUS (MLV) BY THREE DIFFERENT H-2 LINKED IMMUNE RESPONSE GENES. Jean Paul LEVY, Sylvie GISSELBRECHT, Patrice DEBRE, Elisabeth GOMARD - INSERM U152 - Hôpital Cochin - Paris - France

Three different genes mapping on chromosome 17th control viremia after Moloney virus infection : Rmv1 (in K or IA regions), Rmv2 (in IC, S or G) and Rmv3 (in D or T regions). Inoculation of adult mice with inactivated MLV results in the production of high levels of circulating ant-MLV antibodies in resistant strains, while sensitive strains produce lower levels of antibodies. All three Rmv genes are apparently involved in the genetic control of anti-MLV responses. The use of living MLV does not allow to reveal the role of these genes probably due to in vivo absorption of specific antibodies in viremic animals. On the other hand H-2 restricted cell mediated reactions directed against the MLV induced FMR antigen have been studied by the chromium release test : an immune response gene mapping in H-2D region controls the T killer cells (TK) production by regulating the choice of the better available H-2 + FMR antigenic association by TK precursors.

788 PASSIVE IMMUNOTHERAPY: ANTISERUM TREATMENT PREVENTS EXPRESSION OF ENDOGENOUS MOLONEY VIRUS AND AMPLIFICATION OF PROVIRAL DNA IN BALB/Mo MICE, Peter Nobis and Rudolf Jaenisch, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany

BALB/Mo mice carrying the Moloney leukemia virus (= M-MuLV) as an endogenous virus become viremic soon after birth and develop leukemia at a later age. M-MuLV-specific gene expression and an increase of virus-specific DNA copies in lymphatic target organs are characteristic parameters of the preleukemic phase.

Passive immunotherapy of newborn BALB/Mo mice with anti-gp70 or anti-M-MuLV serum prevented viremia and subsequent development of leukemia.

Molecular hybridization experiments showed that both virus-specific genome transcription and virus-specific DNA amplification could be completely suppressed by antiserum treatment. Thus virus-specific RNA concentrations in target organs of immunized BALB/Mo mice of six months or older were as low as in normal BALB/c mice. This is an age at which untreated BALB/Mo mice have already developed malignant lymphoma. Our experiments demonstrate that treatment with antiserum interferes with the early events of virus expression and prevents the subsequent steps leading to leukemia.

789 HAMSTERS,LACKING CLASS I POLYMORPHISM,DEVELOP NON-T CELL-MEDIATED VIRUS IMMUNITY. J.W.Streilein, M.Nelles, J.T.Phillips, W.Duncan, U.Tex.H.Sci.Ctr., Dallas,Texas 75235

Primary responsibility for anti-viral immunity is ascribed to genes of the major histocompatibility complex (MHC): while Class II genes (H-2I) of mice are important in immune regulation, recent attention has focused on Class I genes (H-2K,D) which promote and restrict effector T lymphocytes that kill virus-infected target cells in vitro and protect against lethal virus infection. A relationship between extensive polymorphism for Class I antigens and ability of a species to survive viral epizootics has been postulated.

Syrian hamsters, captured from the wild over a 40 year span and many kilometers apart,display marked polymorphism for Class II (Ia-like) antigens, but no polymorphism for Class I antigens, although putative Class I molecules are expressed on hamster cells. To study the possible relationship between Class I antigenic variation and virus immunity, hamsters were infected with vaccinia virus and their lymphoid cells examined for cytotoxicity against virus infected target cells in vitro. Cytotoxic activity that was found among lymphoid cells of vaccinia-infected hamsters was not genetically restricted as expected from the lack of Class I polymorphism. More importantly, the cytotoxicity was not even T cell in nature! Instead, a fraction of cytotoxic activity was virus-specific, owing to an antibody-dependent cell-mediated process; the remaining cytotoxicity lacked viral specificity, probably effected by NK cells. Thus, hamsters fail to utilize cytotoxic T cell effectors during acute virus infection; instead, they rely upon an antibody dependent system, aided by activated NK cells, implying that immunity against virus infections need not require Class I MHC genes, and, perhaps, that Class I polymorphism co-evolves with a species' virus load.

790 IMMUNOSELECTION OF MEMBRANE MUTANTS OF MuLV INFECTED CELLS, Tom Fitting, Martin Ruta, David Kabat, University of Oregon Health Sciences Center, Portland, OR 97201

MuLV infected cells synthesizing abnormal forms of virus-encoded cell surface antigens have been obtained as stable variants by using an immunoselection technique recently developed in our laboratory. This technique selects for variant cells which escape complement mediated cytolysis in the presence of cytotoxic antisera. Numerous variants have been obtained which lack cell surface antigens related to either env or to gag gene products. One anti-gp70 derived mutant cell line (H-4) lacks cell surface and intracellular gp70 due to a cellular defect in processing of the env gene encoded precursor, gp90. However, the H-4 cells release non-infectious virus particles which contain core proteins and reverse transcriptase, suggesting that gp70 is not required for virus release. Similarly, several anti-gag derived variant cell lines lack surface gag antigens although they contain various intracellular forms of glycosylated gag polyproteins. These variants express gp70 on their surfaces and have mutations in their cellular rather than in their viral genomes. This immunoselection procedure has proven useful for isolation and analysis of cellular and viral mutants with defects that prevent cell surface expression of viral components. Studies are in progress to characterize the genetics, the biological functions and the requisite structures of the MuLV viral proteins which are altered in these processing variants.

791 GENETIC AND IMMUNOLOGICAL ANALYSIS OF ENDOGENOUS MOUSE MAMMARY TUMOR VIRUS, Larry O. Arthur, Richard J. Massey, Gerald Lovinger, Bruce Altrock, and Gerald Schochetman, Frederick Cancer Research Center, Frederick, MD 21701

Certain inbred strains of mice contain highly oncogenic exogenous milk-transmitted mammary tumor virus (MMTV) and genetically transmitted endogenous MMTV. To study the ongenicity of the endogenous MMTV, we established a cell.line producing MMTV from a mammary tumor of a C3H mouse which had been foster-nursed on NIH Swiss mice to remove the highly oncogenic exogenous MMTV. This tissue culture-derived C3Hf MMTV has a low oncogenic potential since inoculation of Balb/c and C3Hf mice with C3Hf virus have given no mammary tumors at the end of one year. Greater than 50% of the mice inoculated with equivalent amounts of C3H MMTV developed mammary tumors in less than one year. The C3Hf MMTV is genetically different from exogenous MMTV of C3H, RIII, and GR mice as revealed by analysis of large oligonucleotides generated by ribonuclease T_1 digestion of their 70S RNAs. Some of the genetic differences apparently reside in the env region since radioimmunoassays for the envelope glycoproteins, gp52 and gp36, clearly demonstrate antigenic polymorphism for all four MMTVs. Antigenic differences between C3Hf MMTV and exogenous MMTVs have not been detected in radioimmunoassays for the major gag-coded protein, p27. Since infection of mammary glands by exogenous MMTV is a prerequisite for oncogenicity, cell surface receptors for MMTV were investigated. Cell surface receptor recognition resides with the gp52 molecule because only anti gp52 serum and monoclonal antibody to gp52 neutralize transforming capacity of a KiSV (C3H MMTV) pseudotype containing gp52 as the surface antigen. Highly oncogenic C3H and GR MMTV bind common cellular receptors since they inhibit transformation by the pseudotype, whereas C3Hf MMTV does not inhibit. The possibility that the greater oncogenicity of the exogenous MMTV may reside with enhanced infectivity will be discussed.

792 MONOCLONAL ANTIBODIES AND NATURAL MOUSE SERA AS PROBES FOR ANTIGENIC POLYMORPHISM AND IDENTIFICATION OF FUNCTIONAL DETERMINANTS ON MOUSE MAMMARY TUMOR VIRUS gp52, Richard Massey, Bruce Altrock, Larry Arthur and Gerald Schochetman, Frederick Cancer Research Center, Frederick, MD 21701

Antigenic polymorphism in the major envelope glycoprotein, gp52, of different strains of mouse mammary tumor virus (MMTV) was studied with monoclonal antibodies and sera from mammary tumor-bearing mice. With these immunologic probes, the functional domains of the gp52 molecule were studied by in vitro neutralization of infectivity, complement-dependent cytolysis, precipitation of ^{125}I-MMTV, and a solid-phase radioimmunoassay. The antigenic determinants could be grouped into three categories. Group I: Group-specific antigenic determinants found on C3H, GR, RIII MMTVs, and C3Hf MMTV, an endogenous virus of C3H mice. These determinants function in precipitation and radioimmunoassays. Group II: Class-specific antigenic determinants shared by some, but not all, MMTVs and detected with monoclonal antibodies and natural sera. These include Subgroup A determinants found on C3H and GR MMTV, and Subgroup B determinants found on RIII and C3Hf MMTV. These determinants are targets for neutralizing, cytotoxic, and precipitating antibodies. Group III: Type-specific determinants, unique to a MMTV strain, have only been identified with monoclonal antibodies. Determinants which are targets for neutralizing antibodies may be recognized by cell surface receptors. Based on cell binding and competition assays, C3H and GR MMTV also share a common cell receptor which does not bind RIII and C3Hf MMTV and correlates with Subgroup A identified antigenically. This grouping, based on immunological properties, is analogous to groupings based on oncogenicities of the viruses in BALB/c mice.

793 DISTINGUISHABLE CLASSES OF MURINE LEUKEMIA VIRUS TRANSFORMATION OF HEMOPOIETIC STEM CELLS IN LONG-TERM BONE MARROW CULTURES ARE RELATED TO RECOMBINANT REGIONS OF THE VIRAL GENOME, Joel S. Greenberger, M.D., Joint Center for Radiation Therapy, Department of Radiation Therapy, Sidney Farber Cancer Institute, Boston, MA. 02115.

Long-term bone marrow cultures from NIH Swiss mice in 25% horse or fetal calf serum supplemented with 10^{-7}M hydrocortisone generate granulocyte-macrophage progenitor cells (CFUc) and pluripotent hematopoietic stem cells (CFUs) for periods exceeding 20 weeks. Infection with long latent period leukemia helper viruses produces dysmyelopoietic morphology of granulocytes but does not generate permanent cell lines. In contrast, Abelson virus or Friend virus (FLV-A, FLV-P, or SFFV from SFFV-Balb nonproducer cells) produces permanent promyelocytic leukemia cell lines. To determine whether long latent period helper viruses produced a first "stage" of granulocytic leukemogenesis, cultures were infected with Rauscher-MuLV or Moloney-MuLV and were grown in the presence of 2 ng/mL phorbol myristate acetate (PMA). Other cultures infected with FLV-A, FLV-P, or A-MuLV (M-MuLV) were also treated with PMA. Control cultures were grown with virus or PMA alone. Infection with FLV-A or A-MuLV (M-MuLV) generated permanently transformed granulocytic leukemia cell line within 8 weeks. There was generation of cell lines earlier in virus-infected cultures treated with PMA. In contrast, long latent period leukemia viruses did not generate permanent cell lines alone or in the presence of PMA. Addition of PMA in the absence of virus produced dysplastic morphology of generated granulocytes and increased the number of pure-macrophage CFUc. These data demonstrate that specific genetic regions of FLV-A or A-MuLV are required for complete transformation of hemopoietic stem cells and that PMA, a tumor promoter, cannot replace these genetic requirements.

794 A STUDY OF AVIAN MYELOBLASTOSIS VIRUS-INDUCED NONPRODUCER MYELOBLASTS IN VIVO, Nancy K. Zeller, Carlo Moscovici, and M. Giovannella Moscovici, Dept. of Pathology, University of Florida Med. School and VA Medical Center, Gainesville, Florida 32610

AMV transformed nonproducer cells (NP) were grown in mass culture and their oncogenic potential was examined in Vivo. Different concentrations of NPs were injected intravenously into chick embryos. Migration of these cells labeled with I^{125} was detected by measuring the radioactivity in the organs of the injected embryos. Survival of the NPs within the embryos as well as in the hatched birds was determined by culturing the yolk sac or bone marrow cells from the injected animals. No overt leukemia was observed unless NP injected embryos or hatched birds were subsequently challenged with a helper virus. The lack of disease in the injected animals observed for over one year suggests that host factors are intervening in controlling NP proliferation.

795 RESTRICTED EXPRESSION OF VIRAL NUCLEIC ACIDS AND PROTEINS IN PRIMATE RETROVIRUS INITIATED HUMAN B-LYMPHOBLAST CULTURES. N.R. Miller,[1] M.S. Reitz,[3] L. Ceccherini Nelli[3] R.Dalla Favera,[3] F. Wong-Staal,[3] V.S. Kalyanaraman,[2] F. Ruscetti,[3] Z. Salahuddin,[2] P.D. Markham,[2] R.C. Gallo[3]; [1]BRL, [2]LBI, [3]NCI, Bethesda, Md.

The exposure of fresh human peripheral blood cells to primate retroviruses of the simian sarcoma-leukemia virus (SiSV/SiSAV)-gibbon ape leukemia virus (GaLV) group results in the initiation of B-lymphoblast cultures at 10 times the spontaneous rate. The cell lines are tumorigenic in nude mice, although they have a normal diploid karotype. Of thirty cultures examined, only three were found to produce infectious virus. The other cultures, while restricting virus production, expressed viral nucleic acids and proteins to varying degrees: some cultures contained complete provirus and expressed viral RNA and some viral proteins: others contained much lower levels of RNA and protein: and some contained no detectable viral information. To determine whether the low levels of viral RNA in some of the cell populations was due to low level expression or to expression in only some cells, the individual cells in eight viral initiated cultures were examined for the presence of SiSV/SiSAV RNA. In a virus-releasing cell line, essentially all the cells were positive; 3-30% of the cells in four cultures and 75% of the cells in a culture after passage through a nude mouse showed viral RNA expression. Two cultures contained less than 0.1% positive cells; and one culture was completely negative for expression of viral RNA. The restricted expression of viral information in these lymphoblast lines affords a model for the effect of retroviruses on human hematopoietic cells and the involvement of retroviruses in the initiation and maintenance of the transformed state.

796 TRANSCRIPTION IN ASV TRANSFORMED MAMMALIAN CELLS AND PHENOTYPIC REVERTANTS. David J.Chiswell and John Wyke, Imperial Cancer Research Fund,London.

ASV transformed mammalian cells occasionally revert to a normal phenotype. We have been investigating the mechanisms by which this reversion occurs. Data will be presented on the pattern of transcription in transformed and morphologically reverted cells. The significance of this data will be discussed in terms of possible mechanisms of reversion.

797 HOST AND VIRAL REGULATION OF FRIEND ERYTHROLEUKEMIA. Tak W. Mak, Marcy MacDonald, and Alan Bernstein. Ontario Cancer Institute, and Department of Medical Biophysics, University of Toronto, Toronto, Canada M4X 1K9.

The susceptibility or resistance of mice to the induction of erythroleukemia by Friend virus is determined by a complex array of interactions between host genes (Fv-2, W, Steel, H-2) and the viral genome. Some of these susceptibility loci were first identified because of their involvement in the regulation of different aspects of normal hemopoiesis (W, Steel). We have examined the effect of these host genes on the regulation of normal hemopoiesis as well as on the erythroleukemia induced by both the anemia- and polycythemia-inducing spleen focus-forming virus ($SFFV_A$ and $SFFV_P$ respectively) variants of Friend virus. These experiments have indicated that (i) Friend virus specific sequences are expressed in normal tissue and that their expression is under tissue control; (ii) the expression of these specific sequences are also under host genetic control (Fv-2, H-2); (iii) $SFFV_A$ and $SFFV_P$ induce dramatically different effects on erythroid progenitor cells; (iv) $SFFV_A$ is regulated by the same host susceptibility genes (W, Sl, Fv-2) that affect $SFFV_P$. The mechanism of resistance of Friend erythroleukemia by these host genes will be discussed in the context of the following mechanisms: a) Inhibition of the replication of the transforming virus directly; b) reduction of the number of target cells available for transformation; c) suppression of the proliferation of infected or transformed target cells; and d) suppression of the transformed cells by the immune response.

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798 REGULATION OF MOLONEY MURINE SARCOMA VIRUS EXPRESSION IN INFECTED CELLS. Michael Graiser and Dino Dina, Department of Genetics, Albert Einstein College of Medicine, Bronx, New York 10461

Several lines of mouse and rat cells infected with MSV have been isolated and characterized. All lines contained an integrated MSV proviral genome and expressed MSV specific information at various levels. A rat line, termed clone $60^{+}H^{-}$ was isolated after injection and tumor formation in a nude mouse and shown to grow in suspension. Selection of revertants that could not grow in spinner cultures was performed by growing the cells in suspension in the presence of ^{3}H-thymidine or FdUr. A large number of anchorage-dependent cells was isolated and independently subcloned. An analysis of the mechanism of reversion of all these cell clones is in progress.

It is expected that reversion may be due to loss of the integrated viral genome or retention of the virus genome with a loss of viral gene expression. A third class revertant representing a continuation of viral gene expression may be detected by determining viral mRNA formation employing the techniques of RNA-RNA hybridization and R loop formation electron microscopy.

799 EFFECT OF INTERFERON ON A RECOMBINANT RETROVIRUS, MINK CELL FOCUS-INDUCING(MCF) VIRUS
Nelson A. Wivel, Nat. Inst. of Health, Bethesda, MD 20205 and Paula M. Pitha, Johns Hopkins University Oncology Center, Baltimore, MD 21205
MCF viruses represent a novel class of murine retrovirus isolated from thymuses of AKR mice; they induce foci in mink lung cells and tryptic peptide analysis of gp70 of several strains suggests recombination between ecotropic and xenotropic MuLV. Interferon treatment (either 300 or 150 u/ml) of SC-1 cells chronically infected with MCF strain AK-13, reduced the amount of infectious virus released into the medium by 100-fold. Assay by reverse transcriptase indicated only a ten-fold reduction in the number of particles, suggesting formation of significant amounts of noninfectious virus. There were no detectable ultrastructural alterations in interferon-treated virions as compared to controls. Exposure to interferon did not change the genomic RNA profile of AK-13 virus. Following removal of interferon from the system there was a progressive increase in amount of infectious virus produced. Virion polypeptides and cellular immunoprecipitates were analyzed by gel electrophoresis; synthesis and processing of viral proteins was studied by radiolabeling and pulse-chase. The results suggest that interferon-mediated inhibition occurs following viral transcription and translation. Thus loss of infectivity may be related to cell membrane changes affecting the late stages of virus assembly and maturation.

800 REGULATION OF MCF VIRUS-INDUCED LYMPHOMA BY AN FV-1 ASSOCIATED GENE, Miles W. Cloyd,
NIH, NIAID, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, MT 59840
The naturally-occurring recombinant murine leukemia viruses (MCF viruses) appear to play important roles in development of spontaneous murine lymphomas. In studying the interactions between host genes and MCF virus, progeny of various crosses between AKR/J (completely susceptible to AKR MCF virus-induced lymphoma) and C58/Lw (not susceptible) mice were inoculated with AKR MCF virus and followed for development of lymphoma. Susceptibility to AKR MCF lymphomagenesis segregated with one gene which was closely linked to the Gpd-1 (and Fv-1) loci of AKR. Since AKR and C58 mice have been shown to possess similar Fv-1^{nn} alleles regulating replication of ecotropic murine leukemia viruses, these results suggest the possibility that the Fv-1 gene region may be more polymorphic than previously realized, which may be important in regulating MCF viruses.

801 RECOMBINATION BETWEEN DELETION MUTANTS OF ROUS SARCOMA VIRUS.
G. Steven Martin and P. H. Duesberg, University of California, Berkeley, CA. 94720.

Quail clones transformed by replication-defective mutants of RSV lacking gag pol, and env genes were superinfected with transformation-defective RSV. Replication- and transformation-competent viruses were recovered. Characterization of the RNA of these rescued viruses indicates that they originate by unequal recombination between the two defective parents, generating a non-defective virus.