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TUMOR VIRUSES AND DIFFERENTIATION

Edward M. Scolnick and Arnold J. Levine, Organizers

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Plenary Sessions

Workshops

Poster Sessions

Gene Reassortment

0522 PHAGE GENES AND BACTERIAL PHENOTYPES, Norton D. Zinder, The Rockefeller University, New York, New York 10021.

Bacteriophage, like all viruses, are little more than bits of infective genetic material. Many phages, although in different ways, can intrude their genomes on a bacterial host. They tend to maintain at least a few of their genes in a functional state. It is not then surprising that cell phenotypes are modified by carrying phage genomes. Typical examples would be the control by phage of somatic antigens in Salmonella, toxins in Corynebacter and Streptococci, and restriction enzymes in E. coli. Some of the genes that "convert" are essential to phage growth, others are superfluous.

The filamentous phages convert their hosts to the Col TolII phenotype (DOC sensitivity, ColEl, E3, K resistance). In addition, the cells become resistant to infection by the male-specific phages. Genetic analyses and gene cloning reveals that the gene II protein (a nuclease involved in DNA replication) when over-produced, confers male phage resistance. Gene II protein is a membrane associated protein. Gene III protein confers both phage resistance and the TolII phenotype. Gene III is an integral membrane protein prior to becoming part of the virion. In both instances, deletion mapping located these effects to the amino-terminus of these proteins. Membrane effects and protein translocation will be discussed.

It may be assumed that phage are a legacy of early attempts at sexuality.

0523 THE SYNTHESIS OF VARIANT SURFACE ANTIGENS IN TRYPANOSOMES IS CONTROLLED BY GENE REASSORTMENT. P.Borst, A.Bernards, L.H.T.Van der Ploeg, P.A.M.Michels, A.Y.C.Liu, T.De Lange, J.C.Boothroyd* and G.A.M.Cross*. Section for Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam (The Netherlands) and *The Wellcome Research Laboratories, Department of Immunochemistry, Langley Court, Beckenham Kent BR3 3BS (United Kingdom)

The African trypanosome Trypanosoma brucei evades the immune response of its host by the sequential synthesis of more than 100 variants of a major surface protein, the Variant Surface Glycoprotein (VSG). Using cloned DNA complementary to each of four VSG mRNAs we have shown [1] that each VSG is encoded in a separate gene, that VSG genes occur in families of related genes with partly homologous 3' halves and that activation of some of these genes is accompanied by a gene duplication and transposition to yield an extra expression-linked copy (ELC). Comparison of sequences of cDNA and the cloned corresponding basic copy (BC) genes, has shown in two cases that the 3'-end of the BC gene is replaced in the recombination that gives rise to the ELC [2]. This proves that the ELC is the transcribed copy. The sequence comparisons indicate that the cross-over can take place anywhere in the last 200 nucleotides of the gene. The requirements of this recombination explain the remarkably high sequence conservation of the 3' untranslated segments of VSG mRNAs [3,4].

From a comparison of the restriction maps of ELCs (which elude cloning) and BCs we infer that 1-2 kb in front of the BC is co-transposed to the expression site. Since the 5' edge of the co-transposed segment of several VSG genes is also homologous, like the 3' edge, gene conversion could be the mechanism of the duplication-transposition. Two recent observations suggest that the transposition may activate transcription by promoter addition: (i) we find that a BC gene does not contain the sequence present at the 5'-end of the mature VSG mRNA; (ii) we find minor transcripts that fit a tentative processing scheme that starts with a transcript covering the entire transposed segment.

One of the VSG genes studied by us and three VSG genes studied by Williams et al. [5] do not seem to be activated by a duplication-transposition. How mutual exclusion of transposition-activated (TA) and non-TA VSG genes operates is still unclear.

1 P.Borst et al., (1981) Cold Spring Harbor Symp.Quant.Biol. 45, 935-943.
2 A.Bernards et al., (1981) Cell 27, in press.
3 J.C.Boothroyd et al., (1981) Nucl.Acids Res. 9, 4735-4743.
4 H.K.Majumder et al., (1981) Nucl.Acids Res. 9, 4745-4753.
5 R.O.Williams et al., (1981) Cold Spring Harbor Symp.Quant.Biol. 45, 945-949.

0524 GENETIC RECOMBINATION IN AVIAN RETROVIRUSES, A. M. Skalka, R. Junghans, L. R. Boone, Roche Institute of Molecular Biology, Nutley, NJ 07110, and J. Leis, Case Western Reserve University, Cleveland, OH 44106.

Avian retroviruses undergo two types of recombinational events, both with relatively high efficiency. The first, "homologous recombination", results in exchange of genetic material between retroviruses. The high frequency of this recombination can be explained by the fact that the closely-associated RNA genomes of this diploid virus can be reverse-transcribed concurrently, and that strand-displacement is a fundamental property of the reverse-transcription reaction. Our EM studies of DNA synthesis in permeabilized virions have revealed novel structures (H-structures) with properties consistent with intermediates in recombination. We have elaborated a specific model to describe recombination which is initiated when single (+) strands displaced during DNA synthesis on one genome are assimilated into transient gaps in the DNA of the second genome in a diploid pair.[1] In this model recombination is a by-product of DNA synthesis and the viral reverse transcriptase therefore assumes a dual role.

Detection of an endonuclease activity associated with reverse transcriptase suggests that this enzyme may be utilized in the second type of retroviral recombination as well.[2] This type, "integrative recombination", involves the breakage and rejoining of viral and host DNAs. Analysis of the structure of integrated and unintegrated viral DNA have defined sites and features which bear striking resemblence to those typical of transposition of prokaryotic and eukaryotic moveable genetic elements.[3,4] We are exploring the biochemistry of retroviral DNA integration and the possible role of reverse transcriptase using recombinant DNA molecules and assay systems which exploit the biochemical and genetic advantages of prokaryotic genomes.

REFERENCES:

1. Junghans, R., Boone, L. and Skalka, A. (1981). Submitted for publication.
2. Golemb et al. (1981) J. Virol. 38:548.
3. Ju, G. and Skalka, A. (1980) Cell 22:379.
4. Hishinuma, F. et al. (1981) Cell 23:155.

Viruses with ONC Genes

0525 ORIGIN, STRUCTURE AND FUNCTION OF THE TRANSFORMING REGION OF THE ABELSON MURINE LEUKEMIA VIRUS. David Baltimore, Jean Wang, Stephen Goff+ and Cary Queen, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

The transforming region, V-abl, of the Abelson murine leukemia virus (A-MuLV) has its origins in a normal gene of the mouse, C-abl. C-abl represents a number of small regions of DNA homologous to V-abl separated by long stretches of DNA that are not homologous to V-abl. We interpret this organization to indicate that V-abl represents a cDNA copy of the exons that compose the C-abl gene and that the large non-homologous regions represent introns that have been excised. V-abl joins the MuLV sequence in the A-MuLV genome at a precise point where there is a 4 base homology between the Moloney murine leukemia virus (M-MuLV) sequence and the C-abl sequence. The joining position appears to be in the center of an exon of the C-abl gene. At the 3'-end the V-abl/M-MuLV joint is again precise but the C-abl sequence has not been completed in that region. The organization of the transcripts of the C-abl gene indicate that the C-abl mRNA (of which there are 2) has 400 bases 5' of the region homologous to V-abl and 2-3 kb 3' of the homologous region. All of these structural facts can be reconciled by assuming that the A-MuLV genome arose by the integration of a M-MuLV genome upstream of the C-abl gene followed by a deletion that brought the M-MuLV sequences in apposition to a C-abl exon. Transcription of this deleted gene from the M-MuLV promoter would then generate an RNA transcript with an M-MuLV 5'-end. The final A-MuLV genome presumably arose by a secondary recombination at the reverse transcription level according to the model of Goldfarb and Weinberg (J. Virol. 38: 136, 1981). In the context of the A-MuLV genome, the V-abl sequences encode a tyrosine-specific protein kinase activity. By expressing the V-abl region in E. coli it has been shown that the kinase activity is intrinsic to the V-abl protein because in E. coli the protein is phosphorylated on a tyrosine residue.

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0526 THE ANATOMY OF FUNCTION IN RETROVIRUS ONCOGENES, J.M. Bishop, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

The oncogenes of avian retroviruses provide means by which cells of different embryological lineages can be transformed to neoplastic phenotypes. Several of these genes (src, fps, yes, and ros) transform only fibroblasts and encode proteins that appear to be tyrosine protein kinases. Others (myc, erb, and myb) transform hematopoietic cells by functions as yet unidentified. All of these genes appear to have derived from "proto-oncogenes" or "cellular oncogenes" found in the genomes of both vertebrate and pre-vertebrate species. I will review: 1.) the use of molecular cloning and nucleotide sequencing to compare viral to cellular oncogenes; 2.) evidence concerning the mechanism by which oncogenes might be "transduced" from the genome of normal cells; 3.) efforts to express cellular oncogenes that have been isolated by molecular cloning and reintroduced into avian or mammalian cells in chimeric vectors; 4.) the role of subcellular localization in the function of the protein encoded by src; 5.) the role of the host cell in modulating the structure and function of the src protein; 6.) the "dose" of the src protein required to transform cells to a neoplastic phenotype; and 7.) the use of site-directed mutagenesis to map domains of function within oncogenes. At stake is the hypothesis that retrovirus oncogenes provide suitable models for "cancer genes" indigenous to normal cells.

Reference: Bishop, J.M.; Retroviruses and Cancer Genes. Adv. Cancer Res., Vol. 37, in press (1982).

0527 AVIAN SARCOMA VIRUSES AND PROTEIN PHOSPHORYLATION, R.L. Erikson, A.P. Arrigo, E. Erikson, R. Cook and J.G. Foulkes, Department of Pathology, University of Colorado School of Medicine, Denver, CO 80262

The Rous sarcoma virus transforming gene product ($pp60^{src}$) and its normal cell homologue both appear to be protein kinases with the capacity to phosphorylate tyrosine residues in various protein substrates. Investigations in several laboratories have shown that at least three other classes of avian sarcoma viruses exist each with a unique transforming gene encoding transforming gene products with associated tyrosine-specific phosphotransferase activity. Some of these transforming gene products have been purified extensively and the nature of their associated enzymatic activities will be discussed. Studies on radiolabeled phosphoproteins in infected cells show that dephosphorylation of phosphotyrosine can occur very rapidly, thus suggesting the existence of a phosphotyrosine phosphatase(s). Progress in the purification of the relevant enzymes and their activities, particularly toward $pp60^{src}$, will be discussed.

To date, one protein of M_r = 34,000 has been shown to fulfill several necessary criteria showing it to be a substrate of $pp60^{src}$ and perhaps of the enzymatic activity associated with transforming gene products encoded by other ASVs as well. Studies on the cellular location of the M_r = 34,000 protein reveal it is strongly associated with RNA and shows many features expected of a messenger RNA ribonucleoprotein. However, no evidence is available at this time that its phosphorylation is essential for the viral transformation process.

0528 TRANSFORMING PROTEINS OF THE AVIAN ACUTE LEUKAEMIA VIRUSES MC29 AND AEV, Michael J. Hayman, Paula J. Enrietto, Gary M. Ramsay, Imperial Cancer Research Fund, London, England, L.N. Payne, Houghton Poultry Reseerch Station, Cambridge, England. Thomas Graf, Hartmut Beug. Deutsches Krebsforschungzentrum 6900 Heidelberg. West Germany.

Avian erythroblastosis (AEV) and avian myelocytomatosis virus strain 29 (MC29) transform haematopoietic cells as well as fibroblasts in vitro, and in vivo they cause acute leukaemias and tumours of non-haematopoietic cells. We have isolated mutants of both AEV and MC29 which are impaired in their ability to transform haematopoietic cells but are still able to transform fibroblasts. In vivo these mutants are altered in their ability to cause tumors. The AEV mutant can still give rise to sarcomas but can no longer cause erythroid leukaemia. On the other hand the MC29 mutants do not give rise to any of the tumor types associated with the wild type MC29 virus. Biochemical analysis of the mutants has located the mutations to the erb region of the AEV mutant and the myc region of the MC29 mutants. Recently, back mutants of the AEV mutant and one of the MC29 mutants have been isolated. These viruses are now able to transform haematopoeitic cells. Data will be presented with regard to the nature of these back mutants and the pathogenicity of the disease they now cause in chickens.

0529 TRANSFORMING GENE OF MOLONEY MOUSE SARCOMA VIRUS, Inder M. Verma, Charles Van Beveren, Jackie Papkoff, Flip Van Straaten, Mei H.-T. Lai and Tony Hunter, Tumor Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, California 92138. Moloney mouse sarcoma virus (Mo-MSV) is a replication defective retrovirus capable of transforming fibroblasts in vitro and inducing neoplasia in vivo. Both the unintegrated and integrated forms of Mo-MSV viral DNAhave been molecularly cloned and found to be biologically active. The Mo-MSV src specific (v-mos^{Mo}) sequences were subcloned and used as a probe to identify and molecularly clone its cellular homologue (c-mos^{Mo}) from uninfected mouse cells. The complete nucleotide sequence of the Mo-MSV genome has been determined (1). It contains 5,389 nucleotides and has coding capacity for the entire gag gene, portions of the pol gene and the entire transforming gene. The NH_2-terminal of v-mos^{Mo} gene product and the env gene of Moloney MLV, the presumptive progenitor of Mo-MSV, share 4 out of 5 amino acids. The complete nucleotide sequence of c-mos^{Mo} has also been determined. Furthermore, the homologous and nonhomologous regions between M-MSV DNA and Mo-MLV DNA have also been identified.

In vitro translation of Mo-MSV virion RNA generates an overlapping set of four proteins of approximately 37K, 33K, 24K and 18K, all of which appear to be read in frame from the predicted v-mos^{Mo} nucleotide sequence (2). In the absence of antisera against v-mos^{Mo} gene product, it was difficult to identify the mos gene product in the transformed cells. Based upon the predicted sequence of v-mos^{Mo} gene product we synthesized peptides corresponding to the COOH-terminal region. Antisera raised against the synthetic peptides precipitated all four in vitro v-mos^{Mo} gene products (3). Furthermore, a 37K doublet can be identified from Mo-MSV transformed producer and nonproducer cells. The 37K mos gene products synthesized in vivo and in vitro are structurally identical. Finally, mos gene specific mRNAs have been identified in both transformed producer and nonproducer cells. No transcripts of c-mos^{Mo} gene have been identified, despite a very extensive survey of a variety of cells. The c-mos gene in normal mouse fibroblasts appears to be extensively methylated and is in DNase I resistant confirmation.

1. Van Beveren, C., Van Straaten, F., Galleshaw, J. A., and Verma, I. M. 1981. Cell 27:97-108.
2. Papkoff, J., Hunter, T., and Beemon, K. 1980. Virology 101:91-108.
3. Papkoff, J., Lai, M. H.-T., Hunter, T., and Verma, I. M. 1981. Cell 27:109-119.

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Stem Cells

0530 THE INFLUENCE OF STEM CELL BEHAVIOR ON THE KINETICS OF CARCINOGENESIS. John Cairns, Department of Microbiology, Harvard School of Public Health, Boston, MA 02115

Nearly all human cancers arise in classes of cells that undergo continuous multiplication and loss throughout adult life. Since carcinogenesis is known to be a process that usually extends over at least 20 or 30 years, it must involve long-lasting changes in the stem cells that maintain such programs of cell replacement. Compared to most of the cells in self-replacing tissues, these stem cells divide rather infrequently and should therefore be rather protected against genetic damage; certainly, this seems to be true for the stem cells responsible for spermatogenesis. We should therefore consider the possibility that carcinogenesis may involve processes that disrupt the normal orderly programs of cell renewal and therefore break down the protected status of stem cells.

Apart from their slow division, the other potentially important property of stem cells in their apparent freedom from the forces of natural selection (1). For example, women preserve a fine level of x-chromosome mosaicism in their tissues throughout adult life, showing that neighboring stem cells are not normally in competition with each other. It seems to be only in the immune system and in cancerous or precancerous conditions that competition and selection are allowed to occur.

(1) Cairns, J. Nature 255:197-200 (1975).

0531 POLYOMA HOST-RANGE MUTANTS AND TERATOCARCINOMA STEM CELLS. Daniel BLANGY, Joël AGHION, Luisa DANDOLO, Françoise MELIN and Hubert PINON, I.R.S.C., BP 8, 94802 Villejuif Cedex.

1) Polyoma host-range (Hr) mutants have been isolated on various embryonal carcinoma (EC) cell lines : PCC4 (1), F9 (2,3,4), LT1, from different wild type strains of polyoma (LP A2 and A3 and SP Toronto). All the mutants which have been sequenced show a common characteristic ; the Hr phenotype results from sequence rearrangements in the non coding region on the late side of the origin of replication. A comparison of the sequences of several mutants selected on F9 cells points out the following features :

- all mutants selected from A3 and SP contain the same base-pair change (A.T to G.C at position 5230)
- out of five mutants selected from A2, only one contains this transition whereas all of them have two insertions, a thymine after position 5185 and an adenine after position 5171. These additions are already present in wild-type A3 and SP Toronto strains.
- most F9 mutants exhibit tandem duplications of sequences including the point mutations.

By contrast, Hr mutants selected on PCC4 exhibit more complex rearrangements including deletions and duplications. Mutants adapted for growth on PCC4 do not develop on F9 cells and vice-versa, and LT1 cells are resistant to infection by both types. We have recently isolated polyoma mutants with unrestricted Hr which can be expressed in all three EC lines. Their sequence rearrangements will be described and compared to those observed in other mutants.

2) We have shown that restriction of wt A2 polyoma in PCC4 and F9 cells results from a block of early transcription, and that the genome modification in host range mutants does not create new 5'ends specifically active in EC cells. Since no large T antigen is produced, we investigated the fate of viral DNA upon infection of EC cells with wt or Hr mutants. wt A2 DNA replicates at 37°C in the three EC lines. This replication therefore proceeds via a T antigen independent mechanism as confirmed by the fact that Ts-a mutants do also replicate efficiently at 39°C in these cells. Double mutants carrying both the Hr and the ts-a mutations have been constructed : at 39°C they replicate as efficiently as strains which carry only the Hr genotype. These results imply that replication of both wt and Hr mutants in EC cells is completely independent on the presence of an active ts-a gene product. Resistance of EC cells to wt Py infection would therefore result from the absence of transcription of the late region due to the lack of early gene products.

(1) Vasseur M. et al., Proc. Nat. Acad. Sci. USA, 77, 1068, (1980)
(2) Katinka M. et al., Nature, 290, 720 (1981)
(3) Fujimura F.K. et al., Cell, 23, 809 (1981)
(4) Sikikawa K. and A.J. Levine, Proc. Nat. Acad. Sci. USA, 78, 1100 (1981)

0532 *CELLULAR ENVIRONMENTS AND DIFFUSIBLE MOLECULES REGULATING HAEMOPOIETIC STEM CELLS AND COMMITTED PROGENITOR CELLS,* *T. Michael Dexter, Elaine Spooncer, George Bazill, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Withington, Manchester, M20 9BX, England.*

Haemopoiesis can be maintained in vitro for periods in excess of 6 months provided that a suitable population of marrow derived adherent cells are present. Extensive work has shown that the marrow adherent cells represent the in vitro counterpart of the marrow stroma in vivo - and provide a privileged (maybe inductive) environment for haemopoietic cell development over an extended time period. During this time, haemopoietic stem cell (CFU-S) self-renewal occurs; and concomitant differentiation ensures a continued proudction of committed progenitor cells of the granulocyte (GM-CFC), erythroid (BFU-E), megakaryocytic (Meg-CFC) and lymphoid compartments.Further amplification and maturation of these cells results in the production of mature granulocytes and megakaryocytes although erythropoiesis and lymphopoiesis are normally inhibited at a primitive level of development. However, addition of an appropriate erythropoietic stimulus leads to sequential development of the erythroid lineage and production of terminally differentiated RBC. This induced erythropoiesis is associated with an inhibition in the production of mature granulocytes, without affecting the production of GM-CFC; and the inhibition in granulocyte development appears to be mediated indirectly via the stromal environment rather than through a direct effect upon the committed granulocyte progenitor cells.

In recent work, we have been attempting to determine the conditions necessary for self-renewal versus differentiation of the pluripotent stem cells and their progeny.Experiments include treatment of the long-term cultures with agents which influence the production of cell-surface associated proteoglycans; analysis of the conditioned media from "steady-state" and regenerating long-term cultures for putative haemopoietic regulators; the ability of cloned, permanently growing, marrow stromal cell lines to support haemopoiesis either by cell-cell contact or production of diffusible molecules; and the addition to the cultures of highly purified regulatory molecules with known activities. The results of these studies will be reported.

Furthermore, we have been analysing the conditions necessary for the survival and proliferation of cloned, progenitor cells which appear to have a granulocytic nature. Cells removed from long-term cultures can readily be established as permanently growing "immortal" cell lines provided that they are continually supplied with a factor produced from a myelomonocytic leukaemia cell line (WEHI-3b)and various other cells. The relevance of these factor-dependent cells in normal haematopoiesis and the characterisation, purification and biological activity of the factor necessary for their maintenance will be discussed.

0533 STEM CELLS IN GASTROINTESTINAL MUCOSA, Christopher S. Potten, Epithelial Kinetics, Paterson Laboratories, Christie Hospital, Manchester M20 9BX.

In the small intestine the villus is the functional component (pole) of the tissue. On this finger-like projection the cells perform their ascribed duties, become senescent and are discarded. Replacement of this cell loss occurs at the opposite 'pole', in hidden bags of cells, the crypts, distributed in the connective tissue at the base of the villi. As a consequence there is a cell movement from crypt to villus. The velocity of this cell migration is about 1 cell position per hour. The cells ultimately responsible for this cell replacement lie at the origin of the cell migration. At a position in the crypt that can be identified. The crypt can be regarded as consisting of a family (or a few families) of cells, i.e. cell lineage(s). The position that a cell occupies within the lineage(s) can thus be related to the position a cell occupies within the tissue (crypt). Using this approach the behaviour of cells at different hierarchical levels, including the ancestral stem cells, can be studied by observing the behaviour of cells at specific positions within the crypt. In this way it can be shown that various properties may be specifically associated with cells of a certain hierarchical status. These include, cell cycle duration, circadian rhythms, thymidine incorporation levels and possibly differences in the way old and new DNA strands are segregated at division. Using various cytotoxic agents, including radiation, the response of cells at different positions can be seen to vary with hierarchical position.

The ability of cells to regenerate a severely depopulated tissue through clonal growth (clonogenicity) is a property commonly taken as part of the capabilities of the stem cells. Clonal regeneration studies in small intestine suggest that stem cells are easily recruited into rapid cell cycles, and that the number of clonogenic cells may be as high as 60 per crypt. This is less than the number of proliferative cells (160), but more than the number of cells representing the origin of the cell migration (a single circumferential ring of about 16 cells). This could be more easily understood if the stem cell compartment itself was hierarchical due to an acquired age structure imposed largely by the spatial constraints within the crypt.

Viral Transformation

0534 GENETIC AND BIOCHEMICAL ANALYSIS OF CYCLIC AMP EFFECTS IN TRANSFORMED CELLS, Michael M. Gottesman, Charles Roth, Nancy Richert and Ira Pastan, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.

Cyclic AMP treatment slows the growth and to some extent reverses the morphological phenotype of transformation of many cultured mammalian cells. To define the mechanisms of these phenomena we have been using a genetic analysis of Chinese Hamster Ovary (CHO) cells which are spontaneously transformed (the parental phenotype) or which are transformed by the Schmidt-Ruppin strain of Rous Sarcoma Virus (RSV). Over 30 independent mutants of our parental CHO line have been isolated after selection for resistance to cAMP induced growth inhibition. Of 7 mutants characterized in detail with respect to their cAMP dependent protein kinase (PK) activity, 5 show major alterations in this activity. We conclude from these studies that cyclic AMP effects on cell growth and shape are mediated by PK activity. We have used these mutants to identify at least 2 substrates for PK in intact CHO cells: a 50K protein of unknown function, and the cytoskeletal protein vimentin, which is the sole intermediate filament protein in CHO cells.

RSV-CHO cells were obtained by using RSV-SR(D) to transform a phenotypically normal non-transformed derivative of CHO cells originally isolated by Pollard and Stanners. RSV-CHO cells are relatively insensitive to the growth inhibitory effects of cAMP despite the fact that $pp60^{src}$ immunoprecipitated from their extracts is clearly phosphorylated in a cAMP dependent manner. This cAMP dependent phosphorylation of $pp60^{src}$ has been demonstrated by showing an increased ratio of P-ser/P-tyr in $pp60^{src}$ when intact cells were treated with cAMP and labeled with ^{32}Pi. We conclude that in intact CHO cells, PK related phosphorylation of $pp60^{src}$ occurs. However, the increased phosphorylation of $pp60^{src}$ caused by cAMP does not alter its ability to maintain the transformed phenotype.

0535 CELLULAR SUBSTRATES FOR VIRALLY CODED TYROSINE PROTEIN KINASES.
Tony Hunter and Jonathan A. Cooper, Tumor Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, California 92138

The transforming protein of RSV, pp60^{src}, is a tyrosine-specific protein kinase. In cells transformed by RSV there is a 5-10 fold increase in the level of phosphotyrosine in cellular protein. Several proteins have been identified which contain increased levels of phosphotyrosine in RSV-transformed cells and which may therefore be putative substrates for pp60^{src}. Using two-dimensional gel electrophoresis, proteins of 46K, 39K and 28K have been found to have elevated levels of phosphotyrosine in transformed chick cells. We have been studying the properties and localization of these proteins in normal and RSV-transformed cells, as well as in cells transformed by other viruses whose transforming proteins have tyrosine protein kinase activity. For this purpose we have purified the 46K and 39K proteins and raised antisera.

The following generalizations can be made about these proteins. The 46K, 39K and 28K proteins are all abundant cytoplasmic proteins (0.3, 0.3 and 0.07% of cell protein respectively). In transformed cells a small fraction of the total population of each protein (5-15%) is phosphorylated on tyrosine and serine. The majority of modified molecules have a single phosphate residue added. The unmodified proteins and their phosphorylated forms are not strongly associated with the cytoskeleton under our detergent extraction conditions, even though pp60^{src} kinase activity is cytoskeletal.

After hypotonic lysis, the distribution of the 39K protein depends on the ionic conditions. It appears that a fraction may be membrane bound. Immunofluorescence staining for the 39K protein of both normal and transformed cells shows a reticular cytoplasmic pattern. The 39K protein is highly conserved, and is found in many differentiated cell types, but is absent from some lymphoid cell lines. It is phosphorylated in fibroblasts transformed by RSV, PRC II virus, FSV, ST-FeSV, Y73 virus and Ab-MuLV, and in A431 cells treated with EGF. The same tyrosine appears to be phosphorylated in each case. The 39K protein is not phosphorylated in cells transformed by SM-FeSV, polyoma, SV40 or chemicals. It does not have the properties of the enzymes GAPDH, GPDH, MDH, LDH or aldolase and we suspect it has a structural function.

The 46K protein appears to exist in at least three charge states in normal cells. The most acidic of these is phosphorylated on serine. In cells transformed by RSV, PRC II virus, FSV and Y73 virus, a new form phosphorylated on tyrosine and serine is found. Both the phosphorylated and nonphosphorylated forms are soluble under all conditions of cell fractionation, not sedimentable at 150,000g. Immunofluorescence staining for the 46K protein reveals a diffuse cytoplasmic location in both normal and transformed cells. There is an antigenically related protein in mammalian cells, but this has not been characterized.

0536 HARVEY AND KIRSTEN SARCOMA VIRUSES AND THE P21 GENE FAMILY,
Douglas R. Lowy,[1] Esther H. Chang,[1] Ronald W. Ellis,[2] Matthew A. Gonda,[3] Thomas Shih,[2] Deborah DeFeo[2] and Edward M. Scolnick,[2] [1]Dermatology Branch and [2]Laboratory of Tumor Virus Genetics, NCI, Bethesda, MD 20205; [3]Biological Carcinogenesis Program, FCRC, Frederick, MD 21701

Harvey (Ha) and Kirsten (Ki) sarcoma viruses are highly oncogenic rat-derived retroviruses whose transforming (onc) gene product is p21. Low levels of a related p21 are found in normal cells of many species, including rodents and humans. The viral sequences which encode p21 (v-Ha-ras and v-Ki-ras, respectively) have been localized genetically and biochemically to 1 kb regions near the 5' end of each viral genome; efficient transformation of NIH 3T3 cells by viral DNA requires that the viral LTR be present upstream from the p21 coding region. Foci of transformed cells which contain high levels of p21 can also be induced by ligation of the viral LTR to a cloned normal rat cellular gene (c-Ha-ras) homologous to v-Ha-ras. V-Ha-ras and v-Ki-ras are related to each other, but under stringent hybridization conditions there is little hybridization between the two v-ras genes. Conversely, each v-ras gene hybridizes to distinct "unique sequence" fragments in restriction endonuclease digested genomic DNAs, suggesting that the p21 genes of normal cells are a gene family. This hypothesis has been confirmed by the molecular cloning from normal human DNA of four independent c-ras restriction endonuclease fragments; two hybridize preferentially to v-Ha-ras and two are more closely related to v-Ki-ras. Their structure, expression, and functional capacities will be discussed in comparison with those of the v-ras and rat c-Ha-ras genes.

Gene Activation and Regulation

0537 DNA METHYLATION AND THE CONTROL OF ENDOGENOUS RETROVIRUS GENE EXPRESSION, R. Eisenman[1], P. Heater[1], H. Robinson[2], K. Conklin[3], J. Coffin[3], G. Goubin[4], G. Cooper[4], and M. Groudine[1]. [1]Hutchinson Cancer Center, Seattle WA 98104; [2]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545; [3]Tufts University School of Medicine Boston, MA 02111; [4]Harvard Medical School, Boston MA 02115.

The endogenous avian retrovirus loci, (ev loci) are present in normal chicken cell DNA as independently segregating alleles which differ in their transcriptional activity and in the nature of their gene products (1). For example the ubiquitous locus ev-1 consists of an apparently complete viral genome but is transcriptionally silent, while ev-2 and ev-3 produce viral RNA and proteins and, for ev-2, infectious virus particles. We have previously shown that ev-1 sequences are highly methylated compared to ev-3 and that ev-3, in contrast to ev-1, is preferentially sensitive to DNase I and contains nuclease hypersensitive sites in its LTRs (2). In addition, transient treatment of ev-1 cells with 5-azacytidine (aza-CR) results in transcriptional activation, hypomethylation, and DNase 1 sensitivity of ev-1 as well as production of a defective virus particle. Cells derived from an embryo which has spontaneously activated ev-1 also show nuclease sensitivity and an altered methylation pattern in a fraction of the proviruses which is not found in non-expressing siblings.

We also found that the ev-2 locus, which encodes an infectious virus (RAV-0), can also be activated by aza-CR to produce high levels of virus. Activation appears permanent and correlates with hypomethylation at ev-2. When unsheared DNA from aza-CR treated ev-2 cells was used in transfection experiments virus production was observed in recipient cells while no production could be measured in cells transfected with DNA derived from untreated cells.

Taken together our results suggest that alterations in the methylation of DNA sequences can lead to changes in chromation conformation and transriptional activity of ev loci. Examination of sperm DNA has revealed that both expressed (ev-3) and silent (ev-1) loci are hypermethylated suggesting that lack of methylation during diffentiation may be involved in later expression.

(1). Hayward, W., Braverman, S., and Astrin, S., Cold Spring Harbor Symp. Quant. Biol. 44, 1111, 1980

(2). Groudine, M., Eisenman, R., and Weintraub, H. Nature 292; 311, 1981

0538 HORMONAL REGULATION OF GENES FUSED TO THE MOUSE MAMMARY TUMOR VIRUS PROMOTER, Gordon Ringold, Alger Chapman, Carol Hall, Deborah Dobson, and Frank Lee, Departments of Pharmacology and Biology, Stanford University, Stanford, Ca. 94305

We have constructed plasmids harboring the mouse mammary tumor virus (MMTV) LTR fused to a cDNA coding for mouse dihydrofolate reductase (dhfr)[1]. These DNAs have been introduced into Chinese hamster ovary cells deficient in dhfr by direct selection for dhfr expression and into mouse 3T6 cells by selecting for a linked gene coding for the E. coli XGPRT gene. In both cases the level of dhfr and its corresponding messenger RNA are increased by treatment of the transformants with dexamethasone, a synthetic glucocorticoid. Differences in absolute levels of expression and hormonal sensitivity have been observed in these cell lines and may reflect cellular factors that govern promoter efficiency and hormonal responsiveness.

Chimeric genes containing the MMTV LTR fused to the E. coli XGPRT gene and the herpes virus TK gene have also been introduced into a variety of rodent cell lines, including mouse L cells and rat HTC cells. In all cases, the production of downstream sequences becomes hormone sensitive when the MMTV promoter is the proper orientation. These constructions are also being used to test the possibility that the steroid-receptor complex may be capable of regulating promoters that are not closely linked to the regulatory region. In addition, deletion mutants generated in vitro are currently being analyzed in order to delineate the specific region(s) within the MMTV LTR that serve as homone regulatory sites. Lastly, plasmid vectors have been constructed in which the MMTV and SV40 early promoters direct the synthesis of E. coli β-galactosidase in mammalian cells. The utility of such plasmids will be described.

The use of selectable markers linked to the MMTV LTR has allowed us to devise procedures for using the mouse dhfr cDNA and the E. coli XGPRT gene as amplifiable markers.[2] We have succeeded in selecting cells that overproduce these gene products 50-500 fold and are now attempting to select cells that overproduce the glucocorticoid receptor.

1. Lee,F., Mulligan,R., Berg,P., and Ringold,G. Glucocorticoids regulate expression of dihydrofolate reductase cDNA in mouse mammary tumor virus chimaeric plasmids. Nature 294:228-232, 1981.

2. Ringold,G., Dieckmann,B., and Lee,F. Co-expression and amplification of dihydrofolate reductase cDNA and the Escherichia coli XGPRT gene in Chinese hamster ovary cells. J. Mol. and Appl. Gen., in press.

0539 GLUCOCORTICOID REGULATION OF A VIRAL ONCOGENE EXPRESSED FROM THE MOUSE MAMMARY TUMOR VIRUS PROMOTER, Gordon L. Hager and Michael C. Ostrowski, Tumor Virus Genetics Lab, National Cancer Institute, Bethesda, Md. 20205.
Recombinant chimeras with the p21 transformation gene from Harvey Murine Sarcoma Virus (HaMuSV) linked to the long terminal repeat (LTR) containing the promoter for mouse mammary tumor virus (MMTV) have been constructed. The expression of the p21 gene product in transformed NIH-3T3 cells obtained by transfection with these constructions is induced by the addition of dexamethasone to the culture medium. The level of p21 specific RNA in these cells can also be shown to be inducible, and these RNA's contain 5' ends identical to those found on normal MMTV transcripts in MMTV-infected cells. Transfer of glucocorticoid regulation to a normally non-regulated gene indicates that MMTV DNA contains a cis-acting hormone regulatory site. Although high levels of the p21 gene product are found in these transfectants, the frequency with which transfectants are recovered is very low (~1 focus/μg DNA), compared with the transfection efficiency for DNA's with the p21 gene linked to its normal murine leukemia virus (MuLV) promoter (>10^3 foci/μg). Reconstruction experiments with fragments of MuLV DNA have accordingly been performed. Addition of a sequence from the MuLV LTR (containing a direct repeat of 73 nucleotides that normally resides 180 bp 5' proximal to the site of MuLV RNA initiation) to the MMTV LTR-p21 hybrid chimeras increases the transfection efficiency to approximately 2-5x10^2 foci/μg. This stimulation only occurs in dexamethasone treated cells, and is sensitive to the position and orientation of the added MuLV fragment. Hybrid MMTV-p21 transcripts remain inducible in these transfectants.

Lymphoid Development

0540 LYMPHOCYTE DIFFERENTIATION AND LYMPHOMAGENESIS, I. L. Weissman, M. McGrath, C. Nottenburg, R. Coffman, R. Rouse, G. Kraal, R. Reichert, M. Gallatin, and E. Butcher, Department of Pathology, Stanford University, Stanford, CA 94305

Lymphocytes are mobile cells which possess multiple classes of receptors necessary for their central roles in immune recognition and response. Both of the major lymphocyte classes (T and B cells) possess clonally-derived receptors for antigens, the expression of which is developmentally regulated. B cells mature in the bone marrow, where their expression of surface immunoglobulin receptors for antigen requires sequential genomic rearrangement of immunoglobulin gene segments encoding heavy chains, then those encoding light chains. Several independent mechanisms exist to ensure that only one of the two homologous alleles at either the heavy chain or the light chain locus are expressed in any particular B lymphocyte. T cells mature in the thymus, where they begin to express receptors (of unknown molecular nature) which enable each T lymphocyte to corecognize foreign antigens and self-molecules, a property which allows them to recognize and respond to antigens mainly on cell surfaces. The maturation and selection of thymocyte precursors of T cells occurs in three successive architectural domains, resulting in the emergence of ~1% of these cells from the thymus fully equipped to home to peripheral lymphoid organs and there recognize antigens.

Both B and T cells possess surface receptors which determine their homing capacity by causing them to bind to postcapillary high endothelial venule (HEV) cells lining lymphoid organs; the bound cells subsequently migrate through these venules into the parenchyma of these lymphoid organs. While most lymphocytes in all species studied possess genetically restricted receptors which allow them to home to peripheral lymph nodes or to Peyer's patches, an organ preference does exist. Peyer's patch HEV bind ~70% B cells and ~30% T cells, while peripheral nodes select ~70% T cells and ~30% B cells. Antigen-activated T and B cells lose HEV receptors while within lymphoid organ germinal centers, but some of their progeny appear to reexpress HEV receptors of nearly absolute organ specificity. Absolute homing is not only a property of these normal lymphocytes; neoplastic lymphoid clones may also possess receptors specific for Peyer's patch HEV or peripheral node HEV. Some monoclonal antibodies to these lymphomas block organ-specific HEV recognition by both malignant and normal lymphocytes.

We have also studied the role of cell-surface receptors on retrovirus-induced leukemias and lymphomas. We have proposed that both B and T lymphomas express antigen-specific receptors for retroviral envelope antigens, and that filling of such receptors imparts a mitogenic signal to these cells. According to this hypothesis, such cells may produce, or be in association with cells which produce their mitogenic retrovirus, and in consequence undergo uncontrolled proliferation. Each T lymphoma indeed expresses retrovirus-specific receptors involved in growth and malignancy of these cells. Some B lymphomas express surface immunoglobulin receptors specific for a retrovirus produced either by them or by other cells in their microenvironment; an idiotypic determinant on at least one of these B cell lymphoma immunoglobulins is expressed also on two T cell lymphomas at or near their retrovirus receptors.

0541 ABELSON-VIRUS - HEMATOPOIETIC CELL INTERACTION, Naomi Rosenberg, Gerald Waneck and David DeGrand, Cancer Research Center and Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111.

Abelson virus (A-MuLV) is a unique leukemia virus that induces a thymus-independent lymphoma in mice. In vitro infection of adult bone marrow cells or fetal liver cells from embryos of day 15-20 of gestation leads to transformation of lymphoblastoid cells. These cells lack most differentiation markers associated with mature T and B lymphocytes. However about 60% of all transformants are phenotypically similar to normal pre-B lymphocytes in that they synthesize cytoplasmic immunoglobulin (Ig) in the form of μ heavy chain. Analysis of the Ig gene structure in a large number of individual transformants further supports their relationship to pre-B cells. All of the isolates have rearranged their μ heavy chain gene but most have not rearranged their light chain genes.

The apparent similarity of most clonally-derived A-MuLV-transformed lymphoid cells has led to speculation that this virus is capable of transforming only certain types of lymphocytes that are at a particular point in differentiation. However, our most recent studies indicate that A-MuLV infection of hematopoietic cells can stimulate the growth of a wider range of cell types depending upon both the source of target cells and the culture conditions used during the initial phase of transformation. A-MuLV infection of cells from early gestation fetal liver stimulates growth and differentiation of erythroid cells while A-MuLV(P160) infection of adult bone marrow cells that have been chronically stimulated with LPS induces continuous growth of surface IgM positive lymphoid cells.

Gene Transfer

0542 TRANSFORMING GENES OF HUMAN TUMOR CELL LINES, Mark J. Murray, Chiaho Shih, John J. Toole, Melissa McCoy, Ben-Zion Shilo and Robert A. Weinberg,Ctr. Cancer Research & Biology Dept. Mass. Inst. Tech. Cambridge Mass. 02129

Several laboratories have demonstrated the ability of human tumor cell DNA to induce transformation upon introduction into mouse fibroblasts. In our laboratory we have concentrated on the DNAs of human tumor cell lines SW480 colonic carcinoma, EJ bladder carcinoma and HL60 promyelocytic leukemia. The human DNAs carrying these transforming sequences can be detected in the recipient mouse cells by virtue of highly repeated Alu sequence blocs which are closely associated with these genes. Use of an Alu-specific sequence probe in the Southern blot procedure allows the visualization of a series of DNA fragments which are invariably associated with each of the transforming sequences being studies.

We have introduced the DNAs of the various transfected cells into lambdaphage vectors and isolated components of the resulting libraries which contain Alu blocs. In this manner, we have isolated molecular clones of portions of colonic carcinoma and a leukemia transforming gene, and the entire biologically active sequence present in the EJ bladder carcinoma DNA. Some of these cloned sequences have been used as probes in Southern and Alwine-Stark (Northern) blot analyses of nucleic acids of normal and tumor cells. The results of these analyses are described here.

0543 ANALYSIS OF CELLULAR TRANSFORMING GENES BY TRANSFECTION, Geoffrey M. Cooper[1], Mary-Ann Lane[1], Dorothea Becker[1], Gerard Goubin[1] and Paul Neiman[2], Sidney Farber Cancer Institute[1] and Department of Pathology[1], Harvard Medical School, Boston, MA 02115 and The Fred Hutchinson Cancer Research Center[2], Seattle, WA 98104

DNAs of a variety of neoplasms induce transformation of NIH 3T3 cells with high efficiencies (0.05-0.5 transformants per μg DNA), indicating that carcinogenesis can involve dominant genetic alterations resulting in the activation of transforming genes which are then detectable by transfection. Neoplasms in which such activated transforming genes have been detected include chicken B-cell lymphomas and a nephroblastoma (1), human bladder carcinomas (2), human and mouse mammary carcinoma (3) and human and mouse B- and T-lymphocyte neoplasms. The transforming genes detected by transfection of DNAs of chicken B-cell lymphomas and mouse mammary carcinomas, which were induced by weakly oncogenic viruses, are not linked to viral DNA sequences (1,3,4), suggesting that indirect activation of cellular transforming genes is involved at some stage of oncogenesis by these viruses. Restriction endonuclease analysis of the transforming genes activated in human and mouse mammary carcinomas (3) and in human and mouse B- and T-lymphocyte neoplasms indicates that specific transforming genes are activated in neoplasms of specific types of differentiated cells.

A biologically active cellular transforming gene has been isolated by molecular cloning from NIH cells transformed by DNA of a chicken B-cell lymphoma. The minimum transforming fragment of this clone is approximately 1.8 Kb and induces transformation of NIH 3T3 cells with efficiencies of 5 x 10^3 transformants per μg of cell DNA. The cloned transforming fragment is homologous to normal chicken DNA sequences which are highly conserved in vertebrate evolution.

(1) Cooper, G.M. and Neiman, P.E. 1980. Nature 287:656-659.
(2) Krontiris, T.G. and Cooper, G.M. 1981. Proc. Natl. Acad. Sci. 78:1181-1184.
(3) Lane, M.A., Sainten, A. and Cooper, G.M. 1981. Proc. Natl. Acad. Sci. 78:5185-5189.
(4) Cooper, G.M. and Neiman, P.E. 1981. Nature 292:857-858.

Membranes and Viral Host Range

0544 INTERACTION OF REOVIRUS AND CELL SURFACE RECEPTOR: A PROPERTY OF A REGION OF THE VIRAL HEMAGGLUTININ, Bernard N. Fields, Dale R. Spriggs, Roderick T. Bronson‡, Stuart J. Burstin, and Ken Kaye. Harvard Medical School, Boston, MA. 02115 and ‡ Tufts School of Veterinary Medicine, Boston, MA. 02111

The reovirus type 3 hemagglutinin (HA) determines the viral tropism to neurons in the central nervous system. In addition, the HA is the viral polypeptide recognized by neutralizing and hemagglutination inhibiting antibodies. To define the structural basis for the functions of the HA, we isolated several anti-HA monoclonal antibodies. We demonstrated that there are distinct functional domains on the HA involved in either hemagglutination or neutralization reactions. One of the monoclonal neutralizing antibodies was used to select reovirus with antigenically altered HA proteins. These variant viruses were then tested for neurovirulence by determining their ability to replicate in mouse brains and cause fatal encephalitis. The variant viruses grew inefficiently in mouse brains and were markedly less virulent than the parental type 3 reovirus. Neuropathological studies showed that the variants and parental viruses were also distinguishable by their induction of neuronal cell injury in distinct regions of the brain. These studies suggest that minor alterations in the reovirus HA can lead to significant changes in the central nervous system tropism and virulence of the virus.

0545 DEVELOPMENTALLY REGULATED HOST GENES INVOLVED IN PARVOVIRUS REPLICATION, Peter Tattersall, Barbara Spalholz, Jessica Bratton and David Ward, Yale University School of Medicine, New Haven, CT 06510.

In order to understand the developmentally regulated cellular factors required for the replication of the teratogenic parvoviruses we have studied the interaction of two allotropic variants of minute virus of mice (MVM) with mouse cells. These two virus strains are serologically identical but are reciprocally restricted in their ability to infect fibroblast and T-lymphocyte cell lines. We have found that both viruses bind to the same specific cell surface receptor whether the virus-host interaction is productive or restrictive. In both types of infection, parental single-stranded DNA is used as a template for the synthesis of the first complementary strand. However, in restrictive hosts, further replication of the viral genome is limited and infection aborts prior to the synthesis of viral capsid antigens, without affecting the viability or growth rate of the infected cells. We have selected MVM-resistant mutants of CHO cells (normally productive for the fibroblast specific virus) which apparently mimic this intracellular restriction of virus replication. These mutants arise spontaneously at a frequency of approximately 10^{-4} in culture and appear to revert at a high frequency in the absence of selection, both perhaps epigenetic events. Attempts to derive similar mutants from A9 cells have demonstrated that, in this case, the most frequent event resulting in resistance to MVM is loss of the cell surface receptor. Studies on the expression of this receptor on teratocarcinoma stem cells induced to differentiate *in vitro* indicate that the receptor is also developmentally regulated.

Cellular Proteins

0546 THE ASSOCIATION OF A CELLULAR PROTEIN, P53, WITH DNA VIRUS TUMOR ANTIGENS, Arnold J. Levine, Leonard Kaplan, Moshe Oren, Nancy Reich, Peter Sarnow, Carolyn Sullivan, Rees Thoms, State University of New York at Stony Brook, School of Medicine, Department of Microbiology, Stony Brook, New York 11794.

A wide variety of transformed cells contain elevated levels of a cellular protein, termed p53, when compared with their nontransformed counterpart (1,2,3). In SV40 transformed cells p53 is detected in a close association with the SV40 large T-antigen, forming a multimeric high molecular weight complex (2,3). The SV40 large T-antigen regulates the levels of p53 in virus infected and transformed cells (4). In nontransformed cells (3T3 cells) p53 is synthesized and the protein has a very short half life (20-60 minutes) keeping the steady state amounts of p53 at low concentrations (5). In contrast, the half life of p53 proteins in SV40 transformed cells is much longer (greater than 22 hours) resulting in higher levels of p53(5). Either an SV40 large T-antigen induced modification of p53 or the physical association of SV40 large T-antigen with p53 appears to reduce the protein turnover of p53 in transformed cells (5).

In adenovirus transformed mouse cells p53 is detected in elevated levels and found to be complexed with the adenovirus E1b-58K tumor antigen (6). These two proteins form a high molecular weight multimeric complex similar to the one detected in SV40 transformed cells. When monoclonal antibodies specific for the p53 antigen in primate cells are utilized to immunoprecipitate this protein from Epstein-Barr Virus transformed human lymphocytes (Raji, Burkitt Lymphoma Cells), p53 is immunoprecipitated and a second protein of 65,000 MW is also detected in these immunoprecipitates. An Epstein-Barr Virus Nuclear Antigen (EBNA) of 65,000 MW has recently been reported (7) and so this brings up the possibility that EBNA is also physically associated with p53 in transformed human cells (8). The possible significance of the association of a cellular protein, p53, with diverse DNA virus tumor antigens is under investigation.

1. DeLeo, A. B., Jay, G., Appella, E., DuBois, G.C., Law, L.W. and Old, L.J. 1979. Proc. Natl. Acad. Sci. USA 76:2420-2424
2. Lane, D. and Crawford, L.V. 1979, Nature 278:261-263
3. Linzer, D.I.H. and Levine, A.J. 1979, Cell 17:43-52
4. Linzer, D.I.H., Maltzman, W. and Levine, A.J. 1979, Virol. 98:308-318
5. Oren, M., Maltzman, W. and Levine, A. J. 1981, Cell and Mol. Biol. 1:101-110
6. Sarnow, P., Ho, Y., Williams, J. and Levine, A.J. 1982, Cell, in press
7. Strnad, B., Schuster, T.C., Hopkins, R.F., Neubauer, R.H. and Rabin, H. 1981. J. Virol. 38:996-1004
8. Luka, J.H., Jornvall, H. and Klein, G. 1980, J. Virol. 35:596-602.

Long Latency Neoplasms

0547 ONCOGENE ACTIVATION BY AVIAN LEUKOSIS VIRUS, William S. Hayward[1], Benjamin G. Neel[2], and Susan M. Astrin[3]. (1) Sloan-Kettering Institute for Cancer Research, New York, NY 10021; (2) Rockefeller University, New York, NY 10021; (3) Institute for Cancer Research, Philadelphia, PA 19111.

Avian leukosis viruses (ALVS) and other slowly transforming retroviruses lack oncogenes. Studies in the ALV system suggest that these viruses exert their oncogenic potential by activating cellular genes. In at least 85% of ALV-induced lymphomas, the provirus is integrated adjacent to the c-*myc* gene, the cellular counterpart of the transforming gene (v-*myc*) of MC-29 virus. Most of the proviruses are oriented such that transcription initiating from the viral promotor, reads into the c-*myc* gene, generating an RNA transcript composed of both viral (U5) and cellular (c-*myc*) sequences.

The coding sequences of c-*myc* are located in two major exons (0.7 - 0.9 kb), separated by an intervening sequence of approximately 1 kb. Smaller exons, located further upstream, may encode portions of the leader sequence of the c-*myc* in RNA. Most of the proviral integrations occurred within an 0.8 kb region upstream from the coding sequences, but downstream from potential cellular promoters that are active in a cell-free transcriptional system.

Participation of other c-*onc* genes in viral and non-viral neoplasms will be discussed.

0548 AVIAN LEUKOSIS VIRUSES TARGET FOR SPECIFIC FORMS OF NON-ACUTE DISEASE. Harriet L. Robinson[1], Philip N. Tsichlis[2], and John M. Coffin[3], Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545[1], National Cancer Institute, Bethesda, MD 20025[2], Tufts University School of Medicine, Boston, MA 02111[3].

The results of in vivo oncogenicity tests indicate that helper viruses obtained from stocks of Bryan Rous sarcoma virus (RSV) cause a high incidence of lymphomas, whereas helper viruses obtained from stocks of Fujinami sarcoma virus and avian myelocytomatosis virus and transformation defective viruses obtained from stocks of Prague-RSV cause a high incidence of osteopetrosis. Evidence will be presented that the ability to cause a high incidence of a specific form of non-acute disease is encoded in the viral genome, is independent of the envelope antigens of the virus, and is not encoded by U3 sequences that promote viral mRNA synthesis.

ALVs that induce a high incidence of lymphoma do so by inserting a provirus adjacent to the host gene c-myc. We speculate that such ALVs encode an integration factor that recognizes the c-myc region of the host genome. According to this speculation, viruses that target for other forms of disease would encode integration factors that recognize regions of the host genome that have the potential for causing these diseases.

Persistency and Latency

0549 STRUCTURES OF INTEGRATED WOODCHUCK HEPATITIS VIRAL DNA'S IN CHRONICALLY INFECTED LIVER AND HEPATOCELLULAR CARCINOMAS. J. Summers, C. W. Ogston, C. Rogler, J. Jonak, S. Astrin, G. Tyler and R. Snyder. Institute for Cancer Research, Philadelphia, PA 19111 and Penrose Research Laboratory, Philadelphia, PA

Woodchuck hepatitis virus (WHV), a hepatitis B-like virus of Eastern woodchucks (Marmota monax) causes persistant infections in wild woodchucks. Naturally infected animals, observed in captivity, develop chronic hepatitis and hepatocellular carcinomas at a high frequency. Hepatocellular carcinomas often contain WHV DNA integrated at one or a few sites per tumor. Integrated viral DNAs from two tumors were cloned, and their structures determined by restriction mapping and heteroduplex electron microscopy. Complex rearrangements in the integrated viral sequences were found, including inversions, duplications and deletions. Only one of the four open reading frames identified by the nucleotide sequence of WHV was found intact in both tumors.

WHV DNA was found to be integrated in the high molecular weight DNA of infected non-tumor hepatocytes. Two recombinant clones containing such integrated DNA were analyzed and found to contain similar rearrangements in the viral sequences. No evidence for specific integration sites on the viral genome was obtained. Three recombinant clones containing high molecular weight inserts consisting totally of viral sequences were analyzed. These forms consisted of sequentially inverted subgenomic segments of viral DNA with no apparent specificity as to the sites of inversion. The inserts were larger than genome length, present at about one copy per cell, and may or may not be derived from integrated DNA.

Poster Session I

0550 CELLULAR PROTEINS REACTIVE WITH MONOCLONAL ANTIBODIES DIRECTED AGAINST SV40 T-ANTIGEN, Lionel Crawford, Keith Leppard, David Lane+ and Ed Harlow, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, +Imperial College of Science and Technology, London SW7 2AZ.

The set of twenty eight monoclonal antibodies, reacting with SV40 large-T antigen has recently been isolated (Harlow et al, 1981, J. Virology 39, 861). Several of these antibodies also react with proteins found in uninfected and untransformed cells. The proteins are different from each other, PAb419 reacting with a 35,000 molecular weight protein, PAb427 with a 75,000 molecular weight phosphoprotein, PAb405 with a 150,000 molecular weight phosphoprotein and PAb204 (3C4, Lane and Hoeffler 1980, Nature 288, 167) with 68,000 molecular weight protein. It is suggested that although some of these cross reactions may be fortuitous they may, as an alternative, reflect similarities of shape and perhaps function between domains of the viral T-antigen and the relevant host proteins.

0551 LACTATE DEHYDROGENASE-ELEVATING VIRUS: A NOVEL MECHANISM OF VIRUS PERSISTENCE. A. Wolstenholme, J.A. Stueckemann, K. Kowalchyk, M.S. Smith, W.J. Swart, W.A. Cafruny, and P.G.W. Plagemann. Dept. Microbiology, Univ. of Minnesota, Minneapolis, MN 55455.

LDV causes a persistent infection in mice, with a lifetime viremia. LDV replication seems to be limited to a subpopulation of macrophages. In primary peritoneal macrophage cultures only 5-15% of the cells are permissive for LDV replication. Electron microscopic studies of these infected cells, and of spleens from infected animals, indicate that the productively infected macrophages die terminating the acute phase of infection. During the subsequent persistent phase the rate of LDV production is less than 1% of that in the initial, acute phase. Persistence in macrophage cultures is dependent on cell growth, as shown by an absolute requirement for macrophage growth factor in the medium. Persistently infected cultures are resistant to superinfection with LDV, although not to MHV (A59). Addition of anti-interferon globulin has no effect on the persistent infection, nor could interferon be detected in the cell supernatants. We also have found no evidence for ts mutants or DI particles in persistently infected mice or cell cultures. We conclude that persistence of LDV infection is due to the virus' specificity for a small subpopulation of macrophages, which is continuously generated by the replication and differentiation of a stem cell. There is some evidence that this specificity is due to a trypsin sensitive receptor on the cell surface. Susceptibility to LDV infection could be a valuable marker in the study of macrophage subpopulations.

0552 METHYLATION OF EPSTEIN-BARR VIRAL DNA IN PRODUCER AND NON-PRODUCER CELLS. Wendy Clough and David Larocca, Molecular Biology, University of Southern California, Los Angeles, 90007

The methylated state of Epstein-Barr viral (EBV) DNA has been compared for EBV-carrying virus producer and non-producer cells. Eight different 5'-CG'-3' containing sites have been examined using restriction endonucleases. Human lymphoblastoid host-cell DNA was shown to be highly methylated at most sites. Southern blotting analysis showed that EB viral DNA was likewise highly methylated at most sites in the non-producer cell line Raji. Viral DNA from producer cells contained two populations in respect to methylation: fully non-methylated viral genomes and highly methylated genomes. The human B cell line EBR represents the conversion of the EBV-negative transformed line Ramos to a non-producer line containing a few copies of the EBV genome per cell. Unlike the non-producer Raji line, the EBV DNA of EBR showed no methylation at any of the sites tested. This association of hypomethylation with a low level of viral gene expression could be related to the fact that this cell line was originally transformed by some agent other than EBV. Also, the parent Ramos cell line is known to contain large amounts of C-type particles and reverse transcriptase. Some interaction between EBV and the retroviruses within this cell line could be responsible for the non-productive state of the hypomethylated herpesvirus genomes.

0553 DNA TRANSFECTION STUDIES OF ENDOGENOUS ECOTROPIC MuLV, James A. McCubrey, Jon M. Horowitz and Rex G. Risser, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

We have studied the infectivity of endogenous murine leukemia virus contained in genomic DNA prepared from the non virus-producing AKR-2B mouse embryo cell line. When non-producer genomic DNA is transfected into NIH-3T3 cells, this DNA is not infectious unless the culture is treated with IdUr, a result previously described by Lowy (P.N.A.S. 75, 5539-5543, 1978). However, when non-producer AKR-2B DNA is transfected into chicken fibroblastic cells, which are then co-cultivated with Sc-1 (mouse) cells (added after washing the transfected culture and a 24-48 hr incubation period), infectious MuLV is recovered. The transfected chicken cell cultures produce ecotropic XC-positive MuLV which is N-tropic.

DNA prepared from thymuses of RF and from non-producing embryo cells from low virus mouse strains are by themselves infectious for chicken cells, however, NIH-3T3 cells require treatment with IdUr for expression of the endogenous proviruses. DNA transfection studies with DNA prepared from other low virus strains (BALB, B6, and CXB Recombinant Inbred Strains) transfected into chicken and mouse fibroblastic cells (BALB, B6, and NFS) will be discussed.

Our studies indicate that nonexpressed MuLV proviruses from several sources are activated on transfection into chick cells. Such studies suggest that the factors governing the expression of MuLV after transfection into NIH-3T3 and chicken cells are different.

0554 Acute and Late Retrovirus Induced Neurologic Diseases, Paul M. Hoffman, Olin M. Pitts, and James M. Powers, VA Medical Center and Medical University of South Carolina, Charleston, SC and Sandra K. Ruscetti, NIH, Bethesda, Md.

Murine sarcoma virus (MSV) and some murine leukemia viruses (MuLV) produce acute (MSV) and late (MuLV) lymphoreticular tumors. The effects of these viruses on the developing nervous system are less well known. We studied neonatal NFS/N mice inoculated (i.c.) with Kirsten or Harvey MSV and Friend MuLV helper virus and found endothelial cells supporting virus replication and undergoing transformation leading to multifocal angioblastomas 10-14 days post-inoculation. A wild mouse ecotropic MuLV (Cas-Br-M) produced a late erythroleukemia and late progressive neurogenic paralysis in neonatally inoculated (i.c.) NFS/N mice. MuLV proliferation occured initially and primarily in endothelial cells with spread to neurons and aberrant replication in them. The late neurologic disease was associated with spongiform encephalopathy similar to that seen in scrapie; however, Cas-Br-M induced disease had high levels of MuLV p-30 and gp-70 in brain while scrapie did not. Proliferation of these retroviruses in endothelial cells appears essential to both acute transformation and late neuronal degeneration, but the role of viral and cellular genes or gene products in each disease is unclear. Studies to elucidate these points are in progress.

0555 SPECIFICALLY UNMETHYLATED CG SITES IN HERPESVIRUS SAIMIRI DNA IN TUMOR CELLS. Ronald C. Desrosiers, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772.

Herpesvirus saimiri (HVS) is indigenous to the squirrel monkey in which it produces no apparent disease; infection of other species of New World primates, however, frequently results in lymphoma and/or leukemia. HVS may be grown permissively in owl monkey kidney (OMK) monolayer cells in culture. Virion DNA produced from lytic infection of OMK cells is not detectably methylated. Viral DNA is methylated in tumor cell lines and in tumor cells taken directly from tumor bearing animals where virus gene expression is limited. The degree of methylation, especially in repetitive DNA (H-DNA), is lower in tumor cell lines able to produce virus than in non-producer lines. Thirteen of 15 HpaII sites and 15 of 17 FnuDII sites were methylated in greater than 90% of the viral DNA molecules in tumor cells taken directly from tumor bearing animals. The remaining four sites, however, were unmethylated in greater than 95% of the viral DNA molecules in tumor cells. The map locations of the four specifically unmethylated sites were identical in the four induced leukemias examined. The non-producer 1670 tumor cell line, in continuous passage for over seven years, contained four similar specifically unmethylated sites. The reproducible consistency of specifically unmethylated sites suggests an important role in the establishment or maintenance of the transformed state. It is presently not known if RNA in tumor cells originates from regions of DNA containing unmethylated sites nor what factors are responsible for the establishment of this particular methylation pattern.

0556 PRODUCTIVE EPSTEIN-BARR VIRUS INFECTION INDUCES A UNIQUE PYRIMIDINE DEOXYNUCLEOSIDE KINASE IN LYMPHOBLASTOID HOST CELLS. Timothy J. Stinchcombe and Wendy Clough, Molecular Biology, University of Southern California, Los Angeles, 90007

We have recently reported that Epstein-Barr virus (EBV) induces the expression of several unique enzymes in cells undergoing productive viral DNA replication. The most recently discovered of these is a pyrimidine deoxynucleoside kinase that is present in the HR-1 producer cell line but not in the EBV positive, nonvirus producer cell line, Raji. When Raji cells are superinfected with EBV, they undergo productive viral DNA replication and we have shown that this kinase activity is induced.

This EBV-induced pyrimidine deoxynucleoside kinase differs from enzymes already present in the lymphocyte host cell in its ability to phosphorylate thymidine, deoxycytidine and bromodeoxycytidine. The virus-induced kinase also differs from cellular kinases in other properties, including rate of nucleoside phosphorylation, enzyme stability, inactivation at 40°C, inhibition by various nucleoside triphosphates and utilization of various nucleoside triphosphates as phosphate donors. We are currently examining the ability of this EBV-induced kinase to phosphorylate nucleoside analogs that function as anti-herpes agents.

0557 Methylation state and transcriptional activity of integrated Moloney murine leukemia proviruses in exogenously infected mouse cells. P.E. Montandon[1], F. Montandon[1], and H. Fan[2]. The Salk Institute, P.O. Box 85800, San Diego, Ca. 92138[1], and Department of Molecular Biology and Biochemistry, University of California, Irvine, Ca. 92717[2].

A cloned line of mouse 3T3 cells productively infected with Moloney murine leukemia virus (M-MuLV), M-MuLV clone A9, was investigated. M-MuLV clone A9 cells contain 10-15 copies of integrated M-MuLV DNA, but DNase I digestion of chromatin followed by solution hybridization suggested that not all M-MuLV proviruses are transcriptionally active (Breindl et. al. 1980). These results were extended by performing limited DNae I digestion of M-MuLV clone A9 cell nuclei, followed by cleavage of extracted DNA with restriction enzymes and blot-transfer hybridization with M-MuLV-specific cDNA. The integrated M-MuLV proviruses were found to reside in chromatin which was of intermediate sensitivity to DNase I. However, only a few proviruses contained sites of DNase I hypersensitivity (generally an indicator of transcriptional activity). These results support the solution hybridization experiments.

The methylation state of the M-MuLV proviruses was also investigated. Restriction endonucleases which have CpG in their recognition sites and which are unable to cleave methylated CpG were used. A number of sites within M-MuLV proviral DNA were tested, and all were found to be unmethylated for most if not all proviral DNA copies. These results suggest that for exogenously acquired M-MuLV proviral copies, lack of methylation might not be correlated with transcriptional activity.

0558 EXPRESSION OF ALEUTIAN DISEASE VIRUS PROTEINS IN TISSUE CULTURE: HOST-CELL RESTRICTION ON VIRUS PRODUCTION IN MINK CELLS, Marshall E. Bloom, NIH, NIAID, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, MT 59840

Although Aleutian disease virus (ADV) induces an immune complex disease and persistent infection in mink, this nondefective parvovirus does not produce infectious virus in mink cells *in vitro*. I have compared ADV replication in a mink cell line (CCL-64) and in the permissive Crandall feline kidney cell line (CRFK). When ADV input multiplicity was adjusted to produce equal numbers of fluorescence positive CRFK and CCL-64 cells, ADV infectivity increased in CRFK (4 logs over input) but failed to increase in CCL-64 over 10 days. However, if intracellular viral protein expression was studied using immunoprecipitation, both cell types permitted synthesis of the 2 major virion proteins (p85 and p75), as well as an ADV-induced nonvirion protein (p71). These data indicated that, although the mink cells (CCL-64) allowed apparently normal expression of ADV protein synthesis, a host cell restriction on complete virus production existed.

0559 *H-2* LINKED GENETIC CONTROL OF GROSS LEUKEMIA VIRUS PRODUCTION, John H. Wolfe, Kenneth J. Blank and Elizabeth Blankenhorn, University of Pennsylvania, Philadelphia PA 19104

Genes linked to the major histocompatibility complex of the mouse (*H-2*) are known to affect susceptibility to spontaneous and gross-virus-induced lymphomagenesis. Studies in our laboratory have shown that a gene(s) linked to *H-2* controls the production of infectious virus by tumor cell lines derived from virally infected mice. Mice carrying the $H\text{-}2^k$ haplotype are susceptible to lymphomagenesis and their transformed cells continually produce infectious virus in long-term tissue culture. Conversely mice of the $H\text{-}2^b$ haplotype are relatively resistant to lymphoma induction and cultured cells derived from high-virus-dose induced tumors stop producing infectious virus after a few passages. Our current studies indicate that in the non-producer cell line a block occurs between the precursor protein product of the viral *gag* gene (pr 65) and at least one of its usual cleavage products, p30 (the major core structural protein).

0560 HIGH MOLECULAR WEIGHT GSHV-SPECIFIC DNA IN CHRONICALLY-INFECTED GROUND SQUIRREL LIVER, Patricia L. Marion, William S. Robinson, Stanford University, Stanford, CA 94305, Charles E. Rogler and Jesse Summers, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.

Ground squirrel hepatitis virus (GSHV) and woodchuck hepatitis virus (WHV) are two closely related members of the hepatitis B or hepadna virus family. They have at least 2 strongly cross-reacting antigens and a high degree of homology between their DNAs and surface antigen polypeptides. But while WHV-bearing adult woodchucks have a very high incidence of hepatomas (41% of 29 animals over a 30 month period), we have observed no liver carcinoma in our colony of 24 GSHV-infected adult ground squirrels after 21 months. As part of a project to explore the apparent differences in oncogenic potential or potentiation of the 2 viruses, we have cloned from an infected squirrel liver several DNA fragments of greater than genome size into a lambdoid vector, using the same conditions under which integrated WHV DNA forms are being studied.

0561 ASYMMETRIC REPLICATION OF VIRAL DNA STRANDS IN LIVER CELLS INFECTED WITH A HEPATITIS B-LIKE VIRUS, W. S. Mason, J. Summers, C. Aldrich and J. M. Taylor, Institute for Cancer Research, Philadelphia, PA 19111

Duck hepatitis B virus (DHBV) is a new member of the family of viruses of which human hepatitis B virus is the prototype. DHBV is found in commercial duck flocks, where it is maintained by vertical transmission through the egg leading to chronic viremia. To study replication of the viral genome, we have isolated DNA from the livers of chronically infected birds. Examination of this DNA by agarose gel electrophoresis and the technique of Southern has revealed relaxed circular DNA, similar to virion DNA, covalently-closed circular DNA, and a heterogeneous population of rapidly migrating species. Examination of the effect of salt concentration on the sedimentation behavior of the viral DNAs indicated a marked sensitivity of the rapidly migrating species, suggesting that these species are largely single-stranded. The electrophoretic mobility of the largest of these was shown to be insensitive to heating to 100°C and similar to that of denatured virus DNA (3 kb), suggesting that this species is a single-stranded copy of the entire virus genome. Hybridization with strand-specific probes prepared by cloning the virus genome into the single-stranded bacteriophage M13 indicated that this and other rapidly migrating species were predominantly minus-strands. The free minus-strand DNA probably represents a precursor to virion DNA, which is known to contain incomplete plus-strands. Results presented in a separate abstract (J. Summers and W. S. Mason) suggest that the template for minus-strand DNA is RNA, and that synthesis of plus-strand DNA from a DNA template occurs as a late step in virus maturation.

0562 EVIDENCE FOR THE HORIZONTAL ACQUISITION OF MURINE AKR VIROGENES BY RECENT HORIZONTAL INFECTION OF THE GERM LINE, Stephen J. O'Brien, Janet L. Moore, Malcolm A. Martin, National Institutes of Health, Bethesda, MD 20205; James E. Womack, Texas A&M, College Station, TX 77843

Several recent reports have established the molecular genetic similarity between the endogenous AKR virus and an N-ecotropic endogenous virus found in the genome of feral Mus musculus molossinus. The similarities are so striking as to suggest a common origin of these viruses, which are present in some, but not all, inbred mouse strains. The virogenes of AKR mice may have been acquired by either: 1) common descent of AKR (and other AKV^+ strains) from a common ancestor of AKR and molossinus animals, or 2) horizontal germ line infection of the AKR strains by molossinus virus at the strain's inception. The sexual descent model carries with it a prediction of relative consanguinity of the AKR strain and molossinus while the horizontal infection model does not. By comparing the composite allozyme genotype of 51 loci from 17 inbred and outbred mouse strains, the "genetic distance" statistic was derived. Genetic distance measures the degree of allelic substitution between populations and increases proportionately with the amount of time the populations have been reproductively isolated. The genetic distance computed between molossinus and AKR is large, nearly 5-10x the distance between known related populations and strains. Cellular DNA sequences which flank the integrated AKV provirus were analyzed by restriction enzyme digestion of liver DNA from molossinus, AKR and additional inbred strains that express ecotropic murine leukemia virus. The integration flanks of 3 AKR provirus sequences were not evident in molossinus cell DNA. These data support the acquisition of virus by horizontal infection of the molossinus virus.

0563 CHARACTERIZATION OF ENDOGENOUS MuLV PROVIRAL LTRs, Arifa S. Khan[1], Joan B. Austin[2] and Malcolm A. Martin[1], [1]Laboratory of Molecular Microbiology and [2]Laboratory of Viral Diseases, NIAID, National Institutes Of Health, Bethesda, Maryland, 20205.

We have analyzed the long terminal repeats (LTRs) associated with 12 endogenous MuLV proviral DNA sequences isolated from a BALB/c mouse embryo DNA library and from shotgun cloning of Eco RI-restricted DNA fragments from adult AKR/J mouse liver DNA. A comparison of the endogenous LTRs with the LTRs of known ecotropic, xenotropic and MCF MuLV proviral DNAs revealed differences in their size and location of several restriction enzyme cleavage sites. The functional activity of one of the endogenous LTRs will be described.

0564 MULV PROVIRUS INTEGRATION AND RNA EXPRESSION IN AKR LYMPHOMAS, Fayth K. Yoshimura and Kathryn Levine, Hutchinson Cancer Research Center, Seattle, WA 98118

Murine leukemia viruses (MuLVs) expressed by the AKR mouse fall into three classes: 1. ecotropic; 2. xenotropic; and 3. recombinant (MCF) MuLVs. We have shown that proviruses of AKR ecotropic MuLV were not amplified in DNA from leukemic thymuses. By molecularly cloning MCF-13 MuLV into λ gt WES . λB, we have identified a 300 bp DNA fragment which recognizes xenotropic MuLV-related sequences. Use of this DNA probe to analyze leukemic tissue DNA by Southern blotting has resulted in the detection of amplified proviral sequences that were not present in DNA from normal tissue. The number of amplified proviruses was small (usually one or two copies) and the sites of integration did not appear to be specific. Analysis of the viral RNAs expressed in leukemic thymuses revealed that there was no expression of AKR ecotropic MuLV RNA. We did detect, however, cellular poly-A containing 38 and 21S RNAs with homology to the 300 bp DNA specific for xenotropic viral sequences. Hybridization of poly-A RNA to a 600 bp fragment specific for the MCF-13 MuLV LTR sequences did not reveal any other sizes of RNA molecules besides those also detectable with the 300 bp probe. A chicken myc-probe did not detect any homologous RNAs from these thymomas. We conclude that recombinant MuLVs are required for infection of target cells and that viral proteins play a role in transformation and not a promoter insertion mechanism.

0565 IMMUNOGLOBULIN GENE REORGANIZATION DURING B CELL DIFFERENTIATION, Robert L. Coffman and Irving L. Weissman, DNAX Research Institute, Palo Alto, CA 94304 and Stanford University Medical Center, Stanford, CA 94305

Cells at two stages of pre-B cell differentiation can be defined in mouse bone marrow with the monoclonal antibody RA3-2C2. Small pre-B cells are the more numerous and are the immediate precursors of surface Ig^+ B cells. Large pre-B cells appear to be the precursors of small pre-B cells. Both pre-B populations synthesize heavy chain but not light chain. The extent of heavy and light chain gene reorganization in these populations was examined by measuring the disappearance of germ-line restriction fragments containing the J_H or J regions relative to germ-line (sperm) DNA. Virtually no J_H genes remained in the germ-line context in either pre-B cell population. In contrast, no significant J rearrangement was found in large pre-B cells only half as much in small pre-B cells as in expressing B cells. Thus, heavy chain rearrangement is a very early event in pre-B differentiation, but light chain rearrangement occurs much later, just prior to surface Ig expression.

0566 NON-RANDOM INTEGRATION OF BABOON ENDOGENOUS VIRUS IN HUMAN CELLS, J.C. Cohen[2], S.J. O'Brien[1], W.G. Nash[1], C.A. Winkler[1], M. Murphy-Corb[2], and R.S. Lemons[1],[1]National Cancer Institute, Frederick, MD 21701, and [2]Tulane Unviersity, School of Medicine, New Orleans, LA 70112. Baboon endogenous virus (BEV) infection of human-hamster hybrid cells has revealed the presence of a genetic locus (Bevi) required for virus replication. In earlier studies this locus was found to be concordant with the human chromosome 6. In this study further genetic analysis was performed to generalize the occurrence of the Bevi locus. Cumulative data for ten distinct parental lines were indicative of the presence of the Bevi locus and concordant with chromosome 6. In addition genetic data was developed which regionally locates the Bevi locus on the short arm. Characterization of the BEV proviral sequences in both human and human-hamster hybrid cells revealed that the virus was integrating at a specific sequence. This sequence was identified by restriction endonuclease digestion and found to be restricted to chromosome 6, although multiple copies of the sequence are present. Furthermore, a BEV-related human sequences was found in these cells and mapped to chromosome 6.

0567 PRENEOPLASIA & MAMMARY CARCINOGENESIS, Trudy Breznik[1], Janet Butel[2], Daniel Medina[2], and Craig Cohen[1], [1]Tulane Medical Center, New Orleans, LA 70112, [2]Baylor College of Medicine, Houston, TX 77030. Preneoplastic and neoplastic tissues from the BALB/c inbred mouse strain were studied for 1) acquisition of new mouse mammary tumor virus (MMTV) proviruses 2) tissue clonality and 3) methylation of proviral sequences. Previous studies have shown acquired MMTV to be hypomethylated while endogenous sequences are extensively methylated indicative of, an inverse correlation between methylation and viral expression. Recently studies of tumors from uninfected animals have revealed that in these tumors demethylation of certain endogenous viral sequences has occurred. Examination of preneoplastic and neoplastic tissues from uninfected and infected animals revealed that while tumors are clonal, preneoplastic tissues are not. In preneoplastic tissues acquisition of new proviruses is observed, however, tumor development represents a subset of the preneoplastic cell population with or without acquired proviruses. Methylation studies reveal that demethylation of endogenous sequences occurs only in transformed tissue suggesting that demethylation may be associated with carcinogenesis. Also that tumors derived from highly infected preneoplastic tissue may maintain only endogenous sequences some of which are specifically demethylated. Finally, when additional proviruses are present in the tumor, demethylation of endogenous sequences is not seen.

0568 ANALYSIS OF T-CELL DIFFERENTIATION ANTIGENS AND ENDOGENOUS MuLV PROTEINS EXPRESSED ON CELLS OF CHEMICALLY INDUCED THYMIC LYMPHOMAS, Maureen M. Goodenow and Frank Lilly, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Percutaneous application of 3-methylcholanthrene (MCA) to RF mice at three months of age results in the development of thymic lymphomas in 100% of the mice within five months. This is in striking contrast with the incidence of spontaneous lymphoma which occurs in approximately 30% of RF mice after one year of age. Expression of T-cell differentiation antigens and endogenous MuLV gag and env gene products on the surface of both MCA-induced tumors and cell-lines derived from these tumors has been analyzed by flow microfluoremetry using a fluorescence-activated cell sorter. Tumors and cell lines exhibit levels of Thy-1, Lyt-1 and Lyt-2 which are lower than those expressed by the major population of normal thymocytes, whereas amounts of H-2 region antigens are significantly higher on most tumor cells than on the majority of normal thymocytes. This low Thy-1 and Lyt-1/high H-2 phenotype of MCA-induced tumors in RF mice is similar to the phenotype of spontaneous lymphomas in AKR mice. While some RF mice express infectious ecotropic virus in the thymus after four months of age, neither xenotropic nor recombinant viruses have been isolated from this strain. No change in this profile of infectious virus production has been observed in MCA-induced tumors. Nevertheless, there is a significant age-associated increase of endogenous viral gp70 and p30 on normal RF thymocytes, similar to AKR. XenCSA, a xenotropic MuLV envelope-related cell surface antigen, increases slightly with age on normal RF thymocytes. MCA-induced tumor cells also exhibit elevated levels of gp70, p30 and XenCSA. Therefore, unlike AKR, there is no increase in infectious MuLV expression associated with increased levels of viral antigens on RF thymocytes or T-cell lymphomas.

0569 Expression of virus-related sequences in carcinogen-induced lymphomas. Eleni Athan, Maureen Goodenow, Frank Lilly and Dino Dina, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

We have examined the structure and transcriptional activity of endogenous virus-related sequences of several lymphoma cell lines from MCA induced thymic tumors. Poly (A) containing RNAs were analyzed by molecular hybridization with probes prepared from the 600 bp terminal repeats (LTR) of Moloney MSV. All cell lines tested express numerous (4-5) and relatively abundant (100 copies/cell) LTR-homologous transcripts. Size and genetic content analysis of these RNAs with probes prepared from the non-oncogenic gag, env, U5, U3 regions of the M-MuLV viral genome has allowed the identification of at least two major RNA classes; 30-40S transcripts carrying U5, gag and U3 information and the 24S carrying U5, env and U3 information. These RNAs represent the expression of partially defective endogenous viral genomes, since no overt viral production has been detected in these cell lines. RNA transcripts found in several cell lines cross-react with a subcloned DNA fragment containing exclusively the xenotropic sequences of the F-SFFV gp52 gene. Our attention has been focused on a third class of mRNAs, about 20S and 16S in size, which are primarily found in two of the cell lines analyzed. These transcripts do not cross-react with any of the virus-specific probes used except with the U5 portion of the LTR sequences. Such mRNAs could result from the fusion of an endogenous LTR to a specific but yet unidentified cellular gene. These cellular sequences carrying the 5'-viral tag have been partially purified by sucrose gradient sedimentation and their complementary DNA has been synthesized in vitro. Molecular cloning of these cDNAs is expected to clarify the nature of the linkage between LTR sequences and the unknown cellular gene(s).

0570 ANTIGENIC VARIANTS OF HERPES SIMPLEX VIRUS SELECTED WITH GLYCOPROTEIN SPECIFIC MONOCLONAL ANTIBODIES, Joseph C. Glorioso, Thomas C. Holland, Steven D. Marlin, Myron Levine, University of Michigan, Ann Arbor, MI 48109.

Herpes simplex virus (HSV) encodes at least four fully processed glycoprotein species designated gC, gB, gD and gE. The glycoproteins largely account for the immunogenicity of the virus and serve as target antigens for neutralizing antibody. In this study, glycoprotein specific monoclonal antibodies were used to demonstrate that HSV undergoes natural antigenic variation at high frequency. HSV-1 glycoprotein specific monoclonal antibodies were produced by polyethylene glycol-mediated fusion of HSV-1 immune Balb/c spleen cells and P3-X63Ag8.653 myeloma cells. Hybrid clones were selected on the basis of production of HSV-1 specific neutralizing antibody. The glycoprotein specificities of the antibodies were determined by immunoprecipitation of radiolabeled viral antigen and SDS-PAGE analysis. Ten hybridomas producing antibodies specific for gC, three for gB, and one for gD have been identified. Two purified antibodies, one specific for gB and one specific for gC, were used to select virus mutants resistant to neutralization by monoclonal antibody plus complement and rabbit anti-mouse immunoglobulin. Escape from neutralization by 85% of the virus in a survivor stock was taken as evidence for resistance to neutralization. The frequency of neutralization resistant antigenic variants in one experiment ranged from 5×10^{-4} in unmutagenized stocks to 1×10^{-2} in mutagenized stocks. Four gC and twenty-one gB variants have been plaque purified. To date, three gC variants have been tested for resistance to neutralization with a panel of six anti-gC monoclonal antibodies. The resulting patterns of resistance have provided evidence for at least two antigenic determinant sites on gC. The data indicate monoclonal antibodies will be useful in defining the antigenic determinants and the frequency of determinant variation of the HSV glycoproteins.

0571 IDENTIFICATION OF ANTIGENIC DETERMINANTS UNIQUE TO THE SURFACES OF CELLS TRANSFORMED BY EPSTEIN-BARR VIRUS, Chris Kintner and Bill Sugden, McArdle Lab., U. of Wisconsin, Madison, WI 53706

Cytotoxic T-cells specific for Epstein-Barr Virus (EBV)-transformed cells have been identified in the blood of patients with infectious mononucleosis (Svedmyr and Jondal, PNAS 72, 1622-1626 (1975)). We have raised hyridomas which secrete monoclonal antibodies specific for the surfaces of EBV-transformed cells with the goal of identifying the targets recognized by the specific, cytotoxic T-cells or of identifying other structures associated uniquely with transformation by EBV. We have isolated four monoclonal antibodies which recognize only EBV-transformed cell surfaces. We have assayed a panel of cells transformed by EBV for their expression of these determinants, termed EBVCS. All cells transformed in vitro express EBVCS determinants uniformly. All EBV-transformed cell lines established from donors with infectious mononucleosis express EBVCS determinants uniformly. Cell lines established from Burkitt's lymphoma patients, however, express EBVCS determinants at reduced or undetectable levels relative to all other EBV-transformed cells tested. If EBVCS determinants are among those recognized by autochthonous, cytotoxic T-cells then the reduced level of EBVCS on the Burkitt's lymphoma cell lines may reflect a selective advantage in vivo for cells which express less of these determinants.

0572 A PROTEIN IS COVALENTLY-BOUND TO THE 5'-END OF THE MINUS DNA STRAND OF THE GENOME OF DUCK HEPATITIS B VIRUS. K.L.Molnar-Kimber, W.S.Mason, and J.M.Taylor, Institute for Cancer Research, 7701 Burholme Ave., Philadelphia, PA 19111.

The small circular genome of Duck Hepatitis B Virus (DHBV), which is similar to Human Hepatitis B virus, consists of two DNA strands: the minus strand is ≈ 3kb and the plus strand varies in length, up to 3kb. We have recently detected a protein which is covalently-bound to the DNA of DHBV as shown by changes in electrophoretic mobility, BND cellulose chromatography, and selective binding to nitrocellulose. The protein-DNA covalent bond is resistant to 0.1M NaOH treatment, implying that the phosphodiester bond involves neither serine nor threoine residues. The following results suggest that the protein is bound to the 5'-end of the minus strand: (i) Restriction enzyme mapping locates the protein-bound fragment between the 5'-end of the minus strand and a Cfo site about 0.5kb away; (ii) Using strand-specific probes shows that the protein is detected on the minus strand only; and (iii) Analysis using BND cellulose chromatography shows that >90% of the protein-bound DNA is minus strand. Preliminary investigations of DHBV-infected liver tissue indicate that the protein is also bound to form II, form III and single strand intermediates.

A protein has been shown to be covalently bound to the DNA and RNA of several distinct classes of viruses, including Human Hepatitis B Virus (Gerlich and Robinson, Cell 21:801-809, 1980). This protein is clearly involved in the initiation of DNA replication of adenoviruses, and in the RNA synthesis of polioviruses. The role of the protein in the replication cycle of DHBV is being investigated.

0573 EXPRESSION OF TWO DISTINGUISHABLE UNLINKED RETROVIRAL LOCI IN DIFFERENTIATING B-CELLS, Christoph Moroni and Jonathan P. Stoye, Friedrich Miescher-Institut, POB 273, CH-4002 Basel, Switzerland.

We are attempting to correlate lymphocyte differentiation stages with the expression of distinct endogenous retroviral loci. Genetic evidence suggests that two unlinked viral loci are activated when B-cells are triggered to mature into Ig-secreting end cells. One locus (Bxv-1) codes for xenotropic virus (BALB virus-II). Low virus release accompanied B-cell differentiation which can be amplified 100-fold by BrdU. This induction locus maps 21 cM from pep-3 on chromosomal one. A second locus (Bdv-1) which becomes activated in differentiating B-cells codes for a defective, non-replicating virus quantifiable by its reverse transcriptase activity. Bdv-1 is induced primarily by LPS, and BrdU amplification is less than twofold. Preliminary experiments suggest that 5-azacytidine is an inducer of Bxv-1, but not of Bdv-1. Experiments are in progress testing whether these loci also become expressed in T-cell growth factor-dependent T-cell lines.

0574 CLONING OF AAV INTO pBR322; RESCUE OF INTACT VIRUS FROM THE RECOMBINANT PLASMID IN HUMAN CELLS

Richard J. Samulski, Kenneth I. Berns, Ming Tan, and Nicholas Myzyczka. Department of Immunology and Medical Microbiology, University of Florida, College of Medicine, Gainesville, Florida.

AAV is a defective parvovirus that requires helper virus co-infection for its own replication (1). In the absence of helper virus, AAV DNA can integrate into the host genome to establish a latent infection. In the cas of latently infected human cells, AAV may be rescued with high efficiency (up to 10% of latent cells yield virus) upon challenge with either adenovirus or herpes simplex virus.

In order to determine essential sequences needed for AAV virion propagation, we have cloned intact duplex AAV DNA into the bacterial plasmid pBR322. Transfection of the recombinant plasmid into human cells with Adenovirus 5 as helper results in rescue and replication of the AAV genome. Rescued virus DNA and wild type AAV DNA are indistinguishable when compared by restriction analysis. The efficient rescue of viable AAV from the recombinant plasmid should facilitate the genetic analysis of AAV. In addition, the recombinant plasmid itself may be a model for studying the rescue of a latent AAV viral infection.

(1) Berns, K.I. and Hauswirth, W.W. Adv. in Virus Res. 25 407-449 (1979).

0575 INFECTION OF MOUSE LYMPHOCYTES BY EPSTEIN-BARR VIRUS (EBV) FOLLOWING TRANSPLANTATION OF EBV-RECEPTORS ONTO THE CELLS. D.J. Volsky[1], I. Shapiro[2]. 1. Dept. of Pathology & Laboratory Medicine, University of Nebraska Medical Center, Omaha, NE 68105. 2. Dept. of Tumor Biology, Karolinska Institute, Stockholm, Sweden. EBV is a transforming virus known to infect only human and marmoset B-lymphocytes or derived cell lines. This unusual host-cell restriction of the virus is due to the inability of EBV to penetrate into cells other than the natural hosts. We developed a method for an efficient overcoming of the plasma membrane barrier against EBV. The procedure is based on implantation of EBV receptors in the receptor-lacking cell membranes, using Sendai virus (SV) envelopes as transplantation vehicles. The implanted receptors are active in binding of EBV and in mediating the virus penetration into formerly nonsusceptible cells as shown by studies utilizing ^{3}H-Thymidine-labelled EBV. The internalized EBV-DNA is biologically active in most of cell types tested, resulting in the induction of EBV-dependent nuclear antigen (EBNA), early antigen (EA), and virus-capsid antigen (VCA). The method was recently applied to EBV-infection of mouse lymphocytes in an attempt to develop a murine experimental system for in vivo studies on EBV-related oncogenesis. It was found that the EBV receptor-implanted mouse lymphocytes were infectable by EBV of both the transforming B-95-8 (B-EBV) and the nontransforming P3HR-1 (P-EBV) substrains. Unlike the natural host, mouse lymphocytes were not readily transformed by B-EBV. Instead, the cells supported a full lytic cycle of the virus, resulting in the production and release of new EBV particles. The virus, designated M-EBV, induced EBNA in EBV-genome-negative cell line Raji and EA and VCA in EBV-DNA-positive Raji cells. The possibility that M-EBV is a new mutant of EBV is now being studied.

0576 DNA SEQUENCE AND IN VITRO TRANSCRIPTION OF PORTIONS OF THE EPSTEIN-BARR VIRUS GENOME,

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We have completed the DNA sequence of a 17 kilobase fragment of the B95-8 strain of Epstein-Barr virus, the Eco RIC fragment, using a modified shotgun sequencing technique. In addition, we have sequenced the region around the terminal repeat of the B95-8 strain of the virus and a portion of the same repeat from the Raji strain. These regions contain multiple large reading frames, several of which correspond well with RNA species reported for EBV transformed cells. To help characterize the transcriptional units from these regions, we have mapped the transcriptional promoters which function in a soluble in vitro system. A number of other interesting DNA features were also found, including several classes of repeated DNA sequences which had not been previously detected. The various repeats showed differing levels of divergence as well as totally different structures.

0577 NUCLEOTIDE SEQUENCING OF RETICULOENDOTHELIOSIS VIRAL GENOME RELATED SEQUENCES.

Gur W. Notani, University of Minnesota, Minneapolis, MN 55455

Reticuloendotheliosis virus strain T (REV-T) is a highly oncogenic avian retrovirus which causes a rapid neoplastic disease of the lymphoreticular system. A transformation defective variant DNA of the REV-T genome, 3,200 base pairs in length, replicating autonomously along with the REV-T and the helper viral genomes in the transformed spleen cell line has been subcloned in the Charon 4A vector (cl, 2-20-4); I. S. Y. Chen, T. W. Mak, J. J. O'Rear, and H. M. Temin, J. Virology, in press). Rev-T sequences in cl, 2-20-4 have been subcloned in the M13mp9 phage and their nucleotide sequencing is in progress. Comparion of these sequences with the REV-T genome and the homologous cellular rel genes will be presented.

0578 SYNTHESIS OF MEASLES VIRUS PROTEINS IN AN SSPE TISSUE CULTURE MODEL SYSTEM, Rachel Sheppard, Cedric Raine, Murray Bornstein and Stephen Udem, Albert Einstein College of Medicine, Bronx, NY 10461

The SSPE-derived, persistently infected measles virus carrier cell line, IP-3-Ca, routinely produces no infectious virus while synthesizing all of the major measles-specific proteins including matrix(M) protein as detected by immunoprecipitation and SDS-PAGE. Matrix protein can be demonstrated following short radiolabel pulses, but not after long labeling periods. Pulse-chase studies reveal that M protein in IP-3-Ca cells turns over more rapidly than does M protein synthesized by BSc-1 cells acutely infected with reference virus, the Edmonston strain of measles. Peptide mapping by partial protease digestion of these radiolabeled M proteins has shown homology between the M protein specified by IP-3-Ca cells and the M protein derived from acutely infected BSc-1 cells. Glycosylation and phosphorylation studies have shown no differences in secondary modifications seen in IP-3-Ca measles proteins and those observed in the polypeptides synthesized during productive infection.

Two infectious virus clones have been isolated from the IP-3-Ca cell line. These isolates specify the synthesis of all the measles proteins including M. Furthermore, this M protein appears to be stable. Our studies have shown that the lesion(s) involved in persistence correlate with the presumed role of M protein in viral maturation. We have observed that a molecular change from an unstable, non-functional M protein to that of a stable, biologically active M protein parallels the transition from a persistent, non-productive infection to an acute, lytic event yielding infectious progeny, suggesting that a mutation in the M gene is responsible for the inability to assemble and release infectious virus particles.

0579 DETECTION OF MULTIPLE EPSTEIN-BARR VIRUS NUCLEAR ANTIGENS BY RADIOIMMUNOELECTROPHORESIS, Tom B. Sculley, Gary R. Pearson and Thomas C. Spelsberg, Department of Cell Biology, Mayo Clinic, Rochester, MN 55905.

We have subjected extracts of EBV-transformed cells to electrophoresis on SDS-polyacrylamide gels, followed by electrophoretic transfer of proteins from the SDS-polyacrylamide gels to nitrocellulose paper and have identified EBNA using antibody and radioiodinated protein A. Two cell lines, NC_{37} and B-95-8, exhibited multiple EBNA components. Antigens with molecular masses of 67 and 70,000 daltons were present in NC_{37} cells, while the B-95-8 cell line contained three antigens with molecular masses of 67, 70 and 73,000 daltons. Raji cells and B-95 transformed Ramos cells each contained single antigens with molecular masses of 70,000 and 73,000 daltons respectively. EBNA was also identified in Ramos cells tranformed by two different strains of EBV. Both cell lines exhibited an EBNA with a molecular mass of 73,000 daltons, suggesting that different strains of EBV may not be the cause of the cell type specific antigens. The 70,000 dalton antigen present in Raji cells has been partially purified by a combination of salt extraction, chromatography on phosphocellulose and Sepharose 6B. This antigen is present in both the cytoplasm and the nucleus,and exhibits an isoelectric point of 5.5. In its native form EBNA behaves as an oligomer with a molecular mass in excess of 200,000 daltons.

0580 REQUIREMENT OF ADENOVIRUS DNA-BINDING PROTEIN AND VAI RNA FOR THE PRODUCTION OF ADENOVIRUS-ASSOCIATED VIRUS POLYPEPTIDES, John E. Janik, Marilyn M. Huston, Kathleen Cho and James A. Rose, National Institutes of Health, Bethesda, MD 20205.

Several early adenovirus (Ad) gene products are required for replication of defective parvoviruses (AAV; Janik et al, 1981, PNAS 78, 1925). Definition of specific helper functions of these factors would provide insight into biochemical details of AAV replication, and, conversely, could help to determine their individual roles in the regulation of Ad macromolecular synthesis. To investigate AAV transcriptional and translational requirements for Ad gene products, a duplex AAV DNA segment (0.03-0.97 map units) was cloned in pBR325 (pLHI). When 293-31 cells were transfected with this plasmid, transcripts were produced which were capable of directing in vitro synthesis of the three AAV structural polypeptides, although in vivo synthesis of AAV polypeptides could not be detected by immunofluorescence or immunoprecipitation. Cotransfection with pLHI and intact Ad DNA enhanced the level of cytoplasmic AAV transcripts, and in vivo synthesis of AAV proteins was readily demonstrable. It was found, however, that combined transfection with pLHI and a recombinant plasmid that contained the Ad DNA-binding protein gene (pDBP) also enhanced the level of cytoplasmic AAV transcripts but did not promote in vivo AAV protein synthesis. When another plasmid that contained the Ad VAI RNA gene (p2VA) was cotransfected with pLHI and pDBP, in vivo AAV polypeptide synthesis occurred. pLHI plus p2VA, however, did not induce in vivo AAV protein synthesis. We conclude that Ad DNA-binding protein augments the level of AAV cytoplasmic transcripts, whereas translation of these transcripts requires Ad VAI RNA.

0581 REGULATION OF HERPES VIRUS GENE TRANSCRIPTION IN VITRO, Thomas W. Beck and Robert L. Millette, Wayne State University School of Medicine, Detroit, MI 48201.
We have used a partially purified preparation of RNA polymerase II from uninfected (Pol II) and HSV-1 infected HEp-2 cells (Pol II-H) to study the transcription of herpes simplex virus type 1 DNA in vitro. The RNA products from whole, native HSV DNA were purified, denatured by glyoxalation, and analyzed by agarose gel electrophoresis. From gel mobilities we calculated weight average chain lengths of 4.00 and 3.84 kb for the Pol II and Pol II-H products, respectively. These values are very close to the average UV target sizes previously determined for the immediate-early (4.07 kb) and early (3.83 kb) gene transcription units. Blot hybridization analyses of the HSV DNA transcripts showed that i) both enzymes transcribed RNA from essentially all regions of the genome, ii) Pol II preferentially transcribed regions coding for the immediate-early mRNAs, and iii) Pol II-H copied preferentially regions coding for the early and late gene products. To further demonstrate promoter selectivity, the two enzymes were used to transcribe the cloned HSV-1 Bam HI-Q fragment (containing the thymidine kinase (TK) gene) and its subfragments. Gel electrophoretic analyses of the products showed the following: i) The transcripts produced by Pol II-H are completely different from those produced by Pol II. ii) Only Pol II-H initiates transcription from the TK promoter. iii) Pol II exhibits little, if any, promoter recognition with this fragment. In contrast to these findings, both Pol II and Pol II-H generated an identical set of transcripts from the adenovirus 2 early region IV. These results demonstrate that this polymerase system can be used to study the regulation of HSV-1 gene transcription in vitro. Further studies of HSV promoter selectivity and the viral specified transcriptional control factors involved will be reported.

0582 LONG TERM CULTURE OF IMMATURE AND MATURE MURINE B LYMPHOCYTES, Cheryl A. Whitlock, Joy Stafford, Alfred Ponticelli, Rosalie Duong, and Owen N. Witte, Dept. of Microbiology and the Molecular Biology Institute, Univ. of California, Los Angeles 90024
Examination of the molecular events involved in differentiation of hematopoietic cells is greatly aided by the development of culture methods that permit enrichment and propagation of the immature precursor cells. We have been successful in designing a culture technique from which we have reproducibly obtained continuously dividing populations that consist of both mature and immature murine B lymphocytes. Bone marrow cells were placed in culture in medium that contained fetal calf serum and no exogenously added steroids. A feeder layer of adherent cells rapidly established which provided an enviroment that supported the growth and development of the bone marrow-derived B lymphocytes for over a year. In such bone marrow cultures, the numbers of cells bearing membrane immunoglobulin increased gradually for four weeks then declined. Between 10 and 14 weeks, several cultures gave rise to continuously dividing B lymphocyte populations that contained pre-B cells (producing mu heavy chains only and sensitive to transformation by Abelson murine leukemia virus) and mature B cells (synthesizing both mu heavy chains and light chains of immunoglobulin). Immunoglobulin molecules synthesized by the continuously dividing populations were heterogeneous by two-dimensional gel analysis. This suggested that mature B cells may have arisen in the cultures via maturation of pre-B cells involving rearrangement and expression of different immunoglobulin variable region gene segments. We are currently investigating the effect of various culture conditions on establishment and growth of continuous B cell lines and are characterizing populations obtained by cloning one of the established B cell cultures.

0583 FELV GAG RELATED SOLUBLE POLYPEPTIDES RELEASED INTO CULTURE MEDIUM BY FESV TRANSFORMED CELLS, Kalyan Ganguly and Max Essex, Dept. Microbiology, Harvard School of Public Health, Boston, MA 02115.
Cat fibroblasts (8-2C) transformed with the Snyder-Theilen strain of feline sarcoma virus release several polypeptides into the culture medium. This cell line was found to be negative for virus production with the reverse transcriptase assay. Four major polypeptides recognized as gag-specific by immunoprecipitation and SDS-PAGE analysis were found in this culture medium. Antisera to gag-proteins p27, p15 and p12 precipitate a glycoprotein of about 46000 daltons from the spent media of ^{3}H-glucosamine-HCl labeled cells. These antisera also precipitate a glycoprotein of about 72000 daltons. In addition to these two glycoproteins, a polypeptide of 30000 daltons is recognized by αp27 serum and polypeptide of about 15000 daltons is recognized by αp12 in medium from cells labeled with ^{35}S-Met for 20 hours. Cat antiserum reactive to p12 also recognize the 15000 dalton polypeptide in spent medium of cells labeled with ^{35}S-Met for the same length of time. When tested for auto-phosphorylation using [γ^{32}-p] ATP, the immunoprecipitate (using αp27 serum) of spent medium indicated a lack of such enzymatic activity. Cultured cat lymphoma cells (FL74) that produce feline leukemia virus (FeLV), do not release the 46000 daltons glycoprotein, however, a glycoprotein of 40000 daltons immunoprecipitable by αFelVp27 is detected in soluble form in culture medium from these cells.

0584 GENERATION OF PROGRAM BLOCKED HEMATOPOIETIC STEM CELLS BY CHEMICAL CARCINOGENS AND TUMOR VIRUSES, Allen E. Silverstone and Louis J. Lafrado, Sloan-Kettering Institute New York, NY 10021

Treatment of murine bone marrow cells in vitro with chemical carcinogens such as 4-nitroquinoline-1-oxide followed by liquid culture on congenic bone marrow feeder layers for 7 weeks at 33°C. yields cell populations and lines that are phenotypically similar to blasts and promyelocytes. These cells retain some differentiation potential for the myeloid series in the presence of WEHI-3 derived colony stimulating activity (CSA), but normal in vitro differentiation to CFU-GM is partially blocked. These lines are either independent of requirement for CSA in vitro, or else dependent on suboptimal (less than 5%) doses of CSA. Comparison of these results with cultures treated with Rauscher or Abelson viruses suggest a distinct target for chemical transformation in vitro in this regime.

A different regime of in vitro carcinogen treatment followed by tumor promoter (TPA) treatment yields cell populations that are enriched in prolymphocytes. These cells are positive for the enzyme terminal deoxynucleotidyl transferase (TdT), but do not express Lyb2 (a surface marker for B and pre-B cells). They are also not inducible for Thy-1 with TP-5. These cells might be program arrested prolymphocytes. Comparison of these results with treatment of bone marrow cells by Abelson leukemia virus followed by liquid cultures suggests a possible common target for this chemical and viral treatment.

0585 SUSCEPTIBILITY TO PRODUCTIVE INFECTION BY THE BOVINE LEUKEMIA VIRUS IS GOVERNED AT THE PROVIRAL LEVEL. James W. Casey and Margery O. Nicolson LSU Medical Center, New Orleans, LA 70112-1393 and The California Institute of Technology, Pasadena, CA 91125

The three BLV proviruses present in a productively infected monoclonal sheep cell line (FLK-BLV) have been cloned into the modified vector L47. These isolates are 8.7kb in length and have identical restriction endonuclease sites. Hybridization patterns obtained from SmaI, XhoI, HpaII and MspI digested FLK-BLV DNA indicate that 2 of the 3 BLV proviruses are extensively modified. Five cell lines, human RD-4, BAT-B88, Dog D17, beagle and guinea pig were infected with BLV at a MOI of approximately 3. In each case no productive infection was observed as monitored by reverse transcriptase activity. However, DNA extracted from these abortively infected cell lines contain an average of 1-2 BLV proviruses per cell. Considering the difficulty we and others have found in establishing productive infection in a wide range of cell lines and the observation that BLV is not expressed in the B lymphocytes of infected cattle, we propose that BLV integrates into domains of host sequences that normally repress its expression.

0586 CHARACTERIZATION OF HUMAN DNA SEQUENCES RELATED TO THE TRANSFORMING AND REVERSE TRANSCRIPTASE GENES OF AVIAN MYELOBLASTOSIS VIRUS, Steven R. Tronick, Carole K. Lengel, Marcel Baluda, and Stuart A. Aaronson, National Cancer Institute, Bethesda, MD 20205, and University of California at Los Angeles, Los Angeles, CA 90024

Avian myeloblastosis virus (AMV) is composed of two distinct sets of sequences, one derived from its naturally associated helper virus (MAV) and the other from chicken cell DNA. The latter sequence (designated amv) appears to be required for viral transformation and is evolutionarily well conserved at low copy number within the genomes of vertebrate species including humans. In order to assess the role of amv sequences in human malignancies, a bacteriophage library of human DNA was screened with a molecularly cloned AMV probe. One recombinant clone hybridized with an amv specific probe, and its reactivity was found to reside in two contiguous Eco RI fragments analogous in size to those previously detected in Eco RI digested human DNA. The human amv-related sequences were found to possess greater homology with the 3' half of the viral amv gene. A second clone failed to hybridize with the amv probe but did react with a MAV-specific probe. These sequences were shown to be present in MAV DNA within a 0.8 kbp fragment localized to the 3' portion of the reverse transcriptase (pol) gene. Studies on whether the pol related sequences represent an endogenous retrovirus or a normal cellular gene will be presented.

0587 PROGRESSIVE MUTATION CAUSES ANTIGENIC VARIATION OF VISNA VIRUS
JANICE E. CLEMENTS, OPENDRA NARAYAN AND SUSAN MOLINEAUX, JOHNS HOPKINS UNIVERSITY, BALTIMORE, MARYLAND 21205

Visna virus, a retrovirus, causes a progressive disease in sheep. The virus persists for the life of the animal despite a humoral immune response. The slow progressive disease caused by the virus may be due to re-occurring acute episodes of viral re-infection brought about by antigenic mutants emerging in the immune host.

Our ongoing studies of the antigenic variants isolated from two independently innoculated animals as well as variants isolated from infected cells in culture maintained in immune serum, indicate a progressive accumulation of mutations in two closely linked regions of the 3' terminus of the viral RNAs. Mapping and sequencing studies done on the viral RNA of the variants indicate that identical changes occurred during antigenic variation in two independent animals, as well as in tissue culture. Based on these studies it appears that antigenic variation if visna virus is a selective phenomenon: while multiple random mutational events probably occur during viral replication only those virus harboring a few very specific genomic changes escape the immunity and survive.

0588 Leader RNA Sequences in LaCrosse Encephalitis Virus
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The genomeof LaCrosse Virus, a member of the Bunyviridae is made up of 3 RNA molecules of approximate weight 2.5, 1.6 and 0.4 x 10^6 daltons. Recently the nucleotide sequences of the termini of the LaCrosse 3 RNA molecules has been reported (Obijeski et al., 1981, NAR 8:2431-2438). Eleven nucleotides at both 5' and 3' termini of all 3 RNAs are conserved and complimentary. We have labelled total nucleocapsid RNA, as well as the separate genome segments at their 3' hydroxyl end with P^{32} and RNA ligase as previously described. These 3' end labels were used as probes to detect leader RNA i.e. short transcripts complimentary to the 3' end of the minus strand genome from cytoplasmic extracts. Different hours of infection have been analyzed for relative amounts of leader RNA. It appears that early infection provides a better source of leader RNA. The function of the leader RNA is at present unknown.

0589 IDENTIFICATION OF SUPERCOILED HBV DNA MOLECULES IN THE LIVER AND SERUM OF CHIMPANZEE PERSISTENT HEPATITIS B VIRUS CARRIERS.
Nelson Ruiz-Opazo, Prasanta R. Chakraborty and David A. Shafritz, Albert Einstein College of Medicine, Bronx, New York 10461

In chimpanzee hepatitis B virus (HBV) carriers, the mechanism of viral persistence has been examined by analyzing the properties of viral DNA molecules in liver and serum. When chimpanzee liver DNA (CH30) was extracted by a hot SDS-phenol procedure, we observed two extrachromosomal HBV molecules migrating on Southern blots at 4.0 kb and 2.3 kb, respectively. These molecules were distinct from Dane particle DNA, which migrated as a heterogeneous band from 2.0-3.25 kb and were converted to linear 3.25 kb full length double stranded HBV DNA on digestion with EcoRl (EcoRl recognizes one site in the HBV genome). Nuclease Sl converted 2.3 kb HBV DNA to 3.25 kb via a 4.0 kb intermediate. Nuclease Bal 31 produced in a similar two stage reaction a 3.25 kb band which was subsequently degraded. The 4.0 kb form of the HBV genome represents a full length nicked relaxed circle and the 2.3 kb band represents the supercoiled form of the HBV genome. We were able to show the presence of supercoiled form of HBV DNA in serum Dane particle preparations by production of the 4.0 kb relaxed circle intermediate upon Sl and Bal 31 nuclease digestion of Dane particle DNA. Persistence of HBV in chimpanzee do not require integration of viral DNA sequences into the host genome. We suggest that most of the Dane particles may represent incomplete viral forms (those containing partially double stranded DNA circles) and that those which contain supercoiled HBV DNA may represent the complete infectious form of Hepatitis B virus. The incomplete forms of HBV might be acting as defective interfering (D.I.) particles and may play a significant role in the establishment and maintenance of the HBV persistent infection.

0590 INTEGRATION OF HBV-DNA INTO LIVER AND HEPATOCELLULAR CARCINOMA CELLS DURING PERSISTENT HBV INFECTION, David A. Shafritz, Albert Einstein Coll. Med., New York, NY 10461

The hepatitis B virus carrier state (persistent HBV infection) is characterized by the presence of viral surface antigen (HBsAg) and virion particles (Dane particles) in the blood. One to 10% of carriers develop chronic liver disease and/or hepatocellular carcinoma. Recent studies, using restriction enzymes, Southern blots and cloned HBV-DNA probes, have demonstrated integrated HBV-DNA in hepatocellular carcinomas and in several human hepatoma cell lines. In hepatoma patients, integrated HBV-DNA has been found in all HBsAg carriers and in several patients demonstrating anti-HBs and anti-HBc in serum, but not HBsAg. Non-tumorous liver also revealed integrated HBV-DNA with the same or a different hybridization pattern from that observed in the tumor. The finding of a unique integration pattern in liver suggests that the bulk of the tissue examined was of monoclonal origin, i.e., was derived from a single cell into which HBV-DNA had become integrated. To explore when integration occurs, carriers of short-term (<2 years) or long-term (>8-10 years) were evaluated. DNA extracts from percutaneous (needle) liver biopsies showed free viral DNA with no specific integration bands in short-term carriers with or without liver disease. In long-term carriers, HBV-DNA was integrated into the host genome with either a diffuse or unique hybridization pattern. HBV-DNA integration correlated with the duration of the carrier state and absence of virions in the serum. HBV-DNA integration was found in several long-term carriers with no histologic evidence of chronic hepatitis (the so called "benign" carrier state). These studies suggest that integration of HBV-DNA occurs during persistent HBV infection irrespective of liver disease and preceeds development of hepatocellular carcinoma.

0591 MuLV RECEPTORS ON SEVERAL MURINE B AND T CELL LYMPHOMAS SHARE IDIOTYPIC DETERMINANTS, Michael S. McGrath, Libuse Jerabek, Glen Tamura, and Irving L. Weissman, Department of Pathology, Stanford University, Stanford, CA 94305

Murine leukemia virus induced T-lymphomas bear surface receptors specific for the leukemogenic virus which induced them. The receptor mediated leukemogenesis hypothesis postulates that continued cell surface antigenic stimulation by retrovirus envelope gene products provides the drive for neoplastic proliferation. To determine the relationship between known immune receptors and malignant cell proliferation, the spontaneous Balb/c B cell lymphoma BCL_1 was investigated. BCL_1 cells bound BCL_1 splenic stromal cell produced retrovirus (BCL_1V) whereas normal spleen cells did not. This binding was mediated through BCL_1 cell surface IgM, which in isolated form bound BCL_1-V whereas three other IgM myeloma proteins, MOPC-104E, CBPC-112, and HPC-76, did not. Rat anti-BCL_1-IgM monoclonal antibodies were prepared which recognized mu chain, cross reactive and BCL_1-specific anti-idiotypic specificities, and MuLV gp70. Detailed analysis of BCL_1-IgM demonstrated that gp70 copurified in an immune complex, and that one apparent cross reactive anti-BCL_1-idiotypic antibody in actuality recognized gp70. Two cross reactive anti-BCL_1 idiotypes were present on several B and T cell lymphomas, and preincubation of these lymphomas with anti-BCL_1-idiotypic antibodies blocked cell surface MuLV binding. Because most B and all T cell lymphomas bound MuLV and many lymphomas shared cross reactive idiotypic determinants, a relationship between BCL_1-IgM and cell surface molecules involved in T lymphoma MuLV recognition is implied. This model is presented as further evidence for the receptor mediated leukemogenesis hypothesis.

0592 Adenovirus and Herpes Simplex Virus Genes Involved in Replication and Rescue of AAV, C. Laughlin, A. Colberg-Poley, N. Jones, G. Ketner, and B. Carter,NIH,Bethesda,Md20205

Replication of adeno-associated virus requires expression of adenovirus or herpes virus genes. We have investigated the roles of Ad early regions 1,3, and 4 by using Ad5 mutants with deletions in region 1a and 1b, and Ad2 mutants with deletions in region 3 or 4. No AAV DNA, RNA,or protein synthesis was detected in KB cells infected with AAV and the region 1 mutants. Quantitative blotting experiments suggest that infection with these Ad mutants resulted in the degradation of most of the infecting AAV genomes. This was not seen in cells infected with AAV alone. An Ad 1b product, and possibly also a 1a product, is required for AAV DNA replication which precedes AAV RNA transcription. The region 3 deletion mutant efficiently helped AAV. The region 4 deletion mutant is a defective helper of AAV: synthesis of AAV RF DNA, SS progeny DNA, capsid protein and infectious particles were decreased to similar extents.Residual AAV replication, (less than 5% of the control) may be due to contaminating wt Ad in the mutant preparations. This together with our previous results indicate that complete AAV replication requires Ad early regions 1,2, and 4. We have developed several lines of latently infected KB cells from which AAV can be rescued by infection with wild type Ad5. We are currently determining which of the Ad early regions are required for rescue. We are also examining AAV rescue by HSV coded functions using novel λ-HSV recombinants.These recombinants,produced by Sau 3AI partial digestion, cover ~80% of the genome with overlapping sequences and include the U_S, U_L, and IR_L/TR_L sequences, the transforming region, and the genes for thymidine kinase, and DNA polymerase.

0593 SYNTHESIS OF THE DNA GENOME OF AN HEPATITIS B-LIKE VIRUS BY REVERSE TRANSCRIPTION OF AN RNA INTERMEDIATE. J. Summers and W. S. Mason, Institute for Cancer Research, Philadelphia, PA 19111

Duck hepatitis B virus (DHBV) is a new member of a family of viruses of which human hepatitis B virus is the prototype. In results presented in a separate abstract, Mason et al. have characterized the viral DNA species found in DHBV-infected liver cells. Their results suggest the presence of large amounts of nascent DNA minus strands free of plus strand template. In order to detect replicative complexes of DHBV DNA we have looked for viral-specific endogenous DNA polymerase activity in homogenates of infected liver. Such activity is found in particles similar to viral cores sedimenting at about 100 S. In contrast to that found in viral cores, the endogenous DNA polymerase reaction from liver-derived cores synthesized both plus and minus viral DNA strands. The synthesis of minus strand viral DNA in these particles differs from that of plus strand DNA in three respects: (i) the minus strand product is predominantly (80-90%) single stranded, while most, if not all, of the plus strand product is double-stranded; (ii) the synthesis of the minus strand is resistant to inhibition by actinomycin D, while synthesis of the plus strand is inhibited; (iii) about 50% of the minus strand DNA is hydrogen bonded to RNA, while all of the plus strand product is hydrogen bonded to minus strand DNA. A model is proposed by which HBV-like viruses replicate through an RNA genome intermediate.

0594 IN VIVO STEM CELL PROLIFERATION, Michael P. Hagan and Daniel Dodgen, AFRRI, Bethesda, Md 20014

Using in vivo BrdUrd-labeling and in vitro near-UV treatment, we have developed a technique for the measurement of cell proliferation in vivo. This technique was first applied to the murine spleen colony-forming unit. For this population of cells, it was confirmed that the majority of cells are not cycling but are committed to cycle in a Poisson manner with an average transition time of once per 52 hr. for B6D2-F1 mice. This work has now been extended to measure the cycling rates of normal C3H strains and of the congenitally anemic Sl/Sl^d mouse strain.

In addition, the agar colony-forming unit ($GM\text{-}CFU_c$) has been assayed in these three mouse strains. This cell population appears to contain at least two kinetically distinct subpopulations, one of which is closely related to the spleen colony-forming unit. In large part, this latter subpopulation is in its first S-phase as a $GM\text{-}CFU_c$. Additionally, this subpopulation was BrdUrd-labeled in the previous cell cycle with kinetics similar to those of the spleen colony-forming unit. For the B6D2-F1 female, this subpopulation of $GM\text{-}CFU_c$ accounts for approximately 30% of the total. Furthermore, treatments which stimulate rapid proliferation of the spleen colony-forming unit also result in a rapid uptake of BrdUrd into this S-phase fraction of $GM\text{-}CFU_c$.

0595 VIRAL DNA IS METHYLATED IN A MODEL SYSTEM OF HERPES SIMPLEX VIRUS LATENCY, C. Mulder[1], H. Youssoufian[1], S.M. Hammer[2], and M.S. Hirsch[2], [1]University of Massachusetts Medical School, Worcester, MA and [2]Massachusetts General Hospital, Boston, MA.

A human lymphoblastoid cell line of T-cell origin, CEM, was infected with Herpes Simplex Virus type I (HSV I), resulting in a persistently infected culture: viral antigens were detected in 1 to 10% of the cells and 1% of the cells produced infectious virus. For a spontaneously occuring interval of about 24 days (day 83 to 106) no virus could be detected. After 850 days in culture the cells have once again stopped producing virus. During these latent periods, virus porduction could be induced by treating the cells with phytohemagglutinin (PHA). In contrast, concanavalin A (Con-A) treated cells remained permanently negative for infectious virus and viral antigens. Thus, the virus in these cells was apparently turned permanently into a latent phase.

During the latent stages of the viral infection, viral genomes could be detected by cleavage of the cellular DNA with restriction endonucleases, transfer of this DNA to DBM-paper after gel-electrophoresis, and hybridization with (^{32}p)viral DNA and cloned DNA fragments. Reconstitution experiments revealed that one to two viral genomes were present per cell in three latent stages of the cell line. Cleavage with endonucleases R.SmaI/XmaI, HpaII/MspI and SalI indicated that the viral DNA in these latently infected cells was heavily methylated in CG dinucleotides. In contrast, during the productive stage ("persistent infection"), the cells contained multiple copies of the viral genome and no methylated sequences could be detected. Experiments are in progress to follow the fate of the viral DNA during the transition from persistent to latent infection and during induction with PHA.

Poster Session II

0596 DEVELOPMENTAL REGULATION OF ABUNDANT LIVER SPECIFIC mRNAs IN THE MOUSE. Richard K. Barth, Kenneth W. Gross, Linda C. Gremke, and Nicholas D. Hastie, Roswell Park Memorial Institute, Buffalo, New York 14263.

The mouse liver contains a group of 10-12 different mRNAs which are each present at about 12,000-15,000 copies per cell. These mRNAs are also highly tissue specific which led us to suspect they were regulated during development. We have isolated and characterized a number of cDNA plasmids containing sequences which correspond to some of the abundant liver mRNAs. Each of the mRNAs these plasmids represent are highly tissue specific, since their concentration in brain, kidney and spleen is several hundred to a thousand fold reduced relative to liver. Four of these mRNAs appear to change in concentration during prenatal development. One of these mRNAs (p54) is not detectable in the liver until one day before birth when it rapidly increases a thousand fold to adult level. Since p54 is under glucocorticoid control in the adult, this hormone may be involved in the developmental regulation of this mRNA. The mRNAs corresponding to the major urinary proteins (MUPs) increase dramatically in concentration during postnatal development. These mRNAs are encoded by 15-20 genes and are also under hormonal control. The MUPs offer an opportunity to study the developmental regulation of a gene family in the mouse. We are currently involved with determining how many MUP genes are transcribed, if the changes in the concentration of MUP and p54 mRNAs are controlled at the transcriptional level, and what role various hormones have in the developmental regulation of these mRNAs. We are also in the process of developing liver cell culture systems in order to facilitate the study of the regulation of expression of the genes these mRNAs represent.

0597 NONLEUKEMOGENIC gp55 STRUCTURAL GENE MUTANTS OF FRIEND AND RAUSCHER SPLEEN FOCUS FORMING VIRUSES. ROLE OF A MEMBRANE GLYCOPROTEIN IN NEOPLASIA, David Kabat, Martin Ruta, Thomas Fitting, Curtis Machida and Richard Bestwick, Oregon Health Sciences University, Portland, Oregon 97201

We have isolated gp55 structural gene mutants of the Friend and Rauscher spleen focus forming viruses (SFFVs) and have obtained evidence that they may be nonleukemogenic or weakly leukemogenic compared with the wild-type SFFVs. The gp55 glycoprotein is metabolized in an unusual heterogeneous fashion - the majority remains in the endoplasmic reticulum and only a small proportion (c.a., 5%) reaches the cell surface. The possible leukemogenic roles of intracellular versus plasma membrane forms of gp55, as evidenced by studies of the mutant glycoproteins, will be described.

The gp55 glycoproteins are structurally and functionally related to the gp70s of dual tropic MCF-type MuLVs. Studies of the processing of wild-type mutant forms of gp70s and gp55s support the following conclusions: (i) There is a slow step in processing of these glycoproteins which occurs in the rough endoplasmic reticulum. (ii) Passage through this barrier from a precursor pool occurs randomly rather than in the cohort order of glycoprotein synthesis. (iii) Transport of membrane glycoproteins from the RER involves binding to carrier(s) which interact with different glycoproteins with different affinities.

0598 PROTEINS ENCODED BY THE LTR OF MMTV, Clive Dickson and Gordon Peters, Imperial Cancer Research Fund, London, England

Retrovirus replication involves the synthesis of a provirus which may integrate at many different sites in the chromosomal DNA of the host. Sequence analysis of several proviral elements shows the provirus to be bounded by long terminal repeats (LTRs) generated from sequences from both the 5' (U5) and 3' (U3) ends of the viral genome RNA. Functional and nucleotide sequence studies implicate LTRs in the regulation of viral, and possibly adjacent host, RNA transcription. Mouse mammary tumor virus is exceptional in that its LTR region contains a substantial open reading frame in addition to the consensus regulatory sequences found in all retrovirus LTRs studied. Identification of this open reading frame (orf) has relied on DNA sequencing data, and in vitro protein synthesis using complementary RNA synthesised from the LTR-DNA cloned into bacterial plasmids. LTRs obtained from different exogenously transmitted MMTV strains and an endogenous provirus all demonstrate the capacity to encode a series of overlapping polypeptides (orf proteins), the largest of which has a MW of about 36K. The conservation of this open reading among different strains of MMTV argues strongly for an in vivo function for this protein(s). To investigate the nature of the orf protein in vivo we are attempting to produce an antiserum by one of two methods: 1) Expression in bacteria of a fusion protein between a known antigen and orf, and; 2) Chemical synthesis of a peptide selected by consulting the known nucleotide sequence for use as an antigen. The progress of these studies will be presented.

0599 USE OF MONOCLONAL ANTIBODIES IN ANALYSIS OF FRIEND MCF VIRUSES ISOLATED FROM MICE INOCULATED WITH FRIEND MURINE LEUKEMIA HELPER VIRUS. Bruce Chesebro, Miles Cloyd and Leonard Evans. Rocky Mountain Laboratories, Hamilton, Montana 59840. Nine mouse monoclonal antibodies reactive with gp70 from Friend MCF virus were derived following immunization with infected cells. These antibodies were tested for reactivity with 9 cloned MCF virus isolates obtained from spleens of leukemic or preleukemic BALB.B mice 1 to 2 months after neonatal inoculation with cloned Friend murine leukemia helper virus (F-MuLV-67). Based on antigens detected by these antibodies, 6 different viruses were identified, including 2 distinct viruses obtained from the same leukemic mouse spleen. Five of the monoclonal antibodies did not react with F-MuLV-67 and appeared to be markers for potential endogenous proviral parents of these MCF viruses. Two of these antibodies failed to react with any of 5 xenotropic MuLV's, which suggested that provirus(es) different from these xenotropic MuLV's were involved in generation of some of the MCF viruses. The striking heterogeneity of MCF viruses observed in this system as early as 1 month after F-MuLV inoculation suggested that there was no selective pressure for the appearance of a single virus-type in leukemic or preleukemic mice. One antigen was detected in all 4 viruses from leukemic mice and in none of 5 viruses from preleukemic mice. However, after inoculation into newborn BALB.B mice none of the 9 MCF viruses tested caused leukemia during an observation period of 4 months. In contrast, inoculation with as little as one XC plaque-forming unit of F-MuLV-67 induced leukemia in the majority of mice within 2-3 months. Thus, there was no direct evidence implying a causative role for any of these MCF viruses in leukemia induction.

0600 EFFECT OF INHIBITORS OF MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION ON CATION TRANSPORT, Ian G. Macara and Lewis C. Cantley, Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138, U.S.A.

We have previously shown that an increase in cell calcium is an essential step in the commitment of murine erythroleukemia (MEL) cells to terminally differentiate (Levenson,R., Cantley,L.& Housman,D. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 5948-5952). A Na/Ca exchange system in the plasma membrane appears to regulate the calcium concentration within the cell. Dimethyl sulfoxide (DMSO), which induces commitment of MEL cells, acts by inhibiting the Na,K-ATPase, thereby increasing cell Na, which in turn increases cell Ca. Several widely different types of drugs are known to block commitment in the presence of DMSO. These include tumor promoters (phorbol esters), glucocorticoids (dexamethasone), local anaesthetics (procaine), and amiloride. The mechanisms by which these drugs block commitment has been investigated in relation to the changes in cation transport described above, in order to elucidate the specific steps in the differentiation program at which they act.

0601 IONIC REGULATION OF THE INITIATION OF MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION. Robert Levenson and David Housman, Department of Biology and Center for Cancer Research, MIT, Cambridge, MA 02139, and Ian Macara and Lewis Cantley, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.
We have been interested in the mechanism by which the level of cytoplasmic calcium ions is regulated during the program of murine erythroleukemia (MEL) cell differentiation. Previous studies (Bridges et al., 1981. JCB 90: 542) have demonstrated that it is the level of calcium ions which apparently serves as a trigger for the initiation of commitment to the terminal erythroid differentiation program. In this study we have used the fluorescent membrane probe $DiOC_6$ to examine the relationship between mitochondrial transmembrane potential, cytoplasmic calcium levels and the initiation of the commitment process. Treatment with DMSO or a variety of other inducers has been found to cause a depolarization of the mitochondrial membrane. This alteration in membrane potential occurs in concert with the initiation of commitment. Agents such as amiloride and EGTA which inhibit commitment and prevent calcium flux also inhibit the DMSO induced depolarization of the mitochondrial membrane. The effect of DMSO on MEL cell mitochondria is mimicked by FCCP, an uncoupler of oxidative phosphorylation. FCCP also causes MEL cell mitochondria to release calcium into the cytoplasm. When MEL cells are treated with DMSO in conjunction with FCCP, commitment is initiated without the long lag period which is normally observed when cells are treated with DMSO alone. These results are consistent with the hypothesis that mitochondrial transmembrane potential plays an important role in the regulation of cytoplasmic calcium levels at the time of commitment of MEL cells to terminal differentiation.

0602 REPOPULATION OF B LYMPHOCYTES IN MICE GRAFTED WITH CELLS FROM LONG TERM BONE MARROW CULTURES, Kenneth Dorshkind and Robert A. Phillips, The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario M4X 1K9 CANADA

B lymphocytes and their immediate precursors are undetectable in long term bone marrow cultures (LTBMC). However, LTBMC cells can differentiate into B cells when grafted into lethally irradiated mice (Jones-Villeneuve, EV and RA Phillips. 1980. Exp Hematol 8:65; Schrader, JW and S Schrader. 1978. J Exp Med 148:823). In order to further define the proliferative and differentiative potential of B cell precursors in LTBMC, the kinetics of B cell reconstitution in irradiated mice grafted with either adherent or nonadherent cells from LTBMC was compared with that of fresh marrow. B lymphocytes were assayed by measuring levels of colony forming B cells (CFU-B). Our data show that: 1) Precursors in both the adherent and nonadherent fractions of LTBMC have substantial B cell repopulating ability; CFU-B are often detectable in spleen and bone marrow by seven days post engraftment. This parallels the rate of reconstitution observed with fresh marrow; 2) Normal levels of CFU-B are attained by four weeks after reconstitution with LTBMC cells if the cultures are young, i.e., 3 weeks post recharge. Cells tested from 6-8 post recharge cultures, while still capable of substantial B cell repopulation, do not consistently reconstitute B cell levels to normal; 3) Cells from the adherent fraction more consistently repopulate B cell levels to normal than do those from the nonadherent fraction. The donor origin of the cells in these experiments have been confirmed by karyotypic analysis with the T6/T6 marker. (Supported by grants from the MRC and NCI of Canada)

0603 NEWLY ISOLATED FRIEND CELL LINES ARE BLOCKED AT THE SAME STAGE OF ERYTHROID DIFFERENTIATION AS ESTABLISHED CLONES, J.-F. Conscience, S. Deslex and F. Fischer, Friedrich Miescher Institute, CH-4002 Basel, Switzerland. Established Friend cell clones (FC) display remarkably similar states of erythroid differentiation, prior to treatment with an inducer, and these represent a block at a late stage of erythropoiesis. 25 new cell lines were isolated by explanting in vitro the spleen and bone marrow cells of DBA/2 mice infected 3 to 8 weeks earlier with Friend virus (FV-P). In addition, 50 clones were obtained from one of these lines. These cells were all characterized, shortly after their isolation, using indirect immunofluorescent staining for spectrin, carbonic anhydrase II and hemoglobin, as well as a set of enzyme activities. All the new cell lines and clones expressed levels of these markers which were very similar to those found in established FC. From this, we conclude that (1) newly isolated, FV-P transformed, cell lines are blocked at the same stage of erythroid differentiation as established FC, (2) this does not result from long term in vitro selection for the appropriate cell type, (3) the constitutive expression of several erythroid markers is very stable in culture and (4) this contrasts with the capacity of these cells to undergo terminal differentiation, either spontaneously or upon induction, which varies considerably with time and from clone to clone.

0604 INFECTION OF MURINE EMBRYONAL CARCINOMA CELL MUTANTS WITH SV40. Makoto Taketo*, Peter A. McCue & Michael I. Sherman. Roche Inst. of Mol. Biol., Nutley NJ 07110.

Undifferentiated murine embryonal carcinoma (EC) cells infected with SV40 do not express early viral genes although adsorption, penetration and uncoating of the virus and transfer of its genome into the nucleus take place [Schwartzendruber, D.E. et al., J. Cell. Physiol. 93, 25-30 (1977)]. EC cells can be induced to differentiate in vitro by exposure to retinoic acid [Strickland, S. & Mahdavi, V., Cell 15, 393-403 (1978)] or hexamethylenebisacetamide (HMBA) [Speers, W.C. et al., Amer. J. Pathol. 97, 563-584 (1979)]; once differentiated, the cells are capable of expressing SV40 antigens following infection [Schwartzendruber, D.E. & Lehman, J.M., J. Cell. Physiol., 85, 179-188 (1975)]. A number of EC mutant lines have been selected for their inability to differentiate in response to either retinoic acid or HMBA [Schindler, J. et al., Proc. Natl. Acad. Sci. USA 78, 1077-1080 (1981); McCue, P.A. et al., in preparation]. These mutants can be classified into several groups based upon ability to differentiate in response to inducers other than the one used in their selection, extent of differentiation in tumor form and presence of cellular retinoic acid binding protein activity. It is conceivable that the mutant EC lines vary in phenotype because some are arrested early in the differentiative sequence whereas others are blocked at later stages. If this is so, then these mutants might respond in different ways to viral infection. To investigate this possibility, the mutant lines are being tested for expression of T antigen following SV40 infection.

0605 GENETIC ANALYSIS OF MEMBRANE IGM, Carol H. Sibley and Paul E. Mains. Department of Genetics, University of Washington, Seattle, WA. 98195

It has recently been established that the membrane and secreted forms of mouse μ chains are identical up to the end of the fourth C region domain, but differ in their extreme carboxyterminus. The secreted form (μ_s) has a 20 amino acid hydrophilic segment which contains the cysteine critical for pentamer formation, while the membrane form (μ_m) has a 41 amino acid segment containing a hydrophobic core presumably critical for membrane interaction. The mRNAs which encode these two forms can be derived by alternative processing of the same primary transcript. We have used cultured mouse B lymphomas as models to study the control of transcription, translation and post-translational modification of μ_m. We have selected membrane IgM^- ($mIgM^-$) cells from clones of $mIgM^+$ 70Z/3, WEHI 231 and WEHI 279.1 cells. For example, the wild-type 70Z/3 cells are $mIgM^-$, but can be induced to become $mIgM^+$ after incubation with Salmonella typhosa lipopolysaccharide (LPS) for 10 hours. The mutant cells fail to become $mIgM^+$ after LPS treatment. These mutants have retained both μ and κ genes, and make intracellular μ chains, but have lost the ability to respond to LPS and become $mIgM^+$. Determination of the number of complementation groups which produce this phenotype will yield information on the control of the inductive process. Characterization of these mutants provides information on the steps critical to expression of μ_m and the interactions with other cellular elements critical to its expression as an external membrane protein.

0606 ESTABLISHMENT OF A LONG-TERM HEMATOPOIETIC STEM CELL CULTURE FROM A MOUSE EMBRYO Suraiya Rasheed, and Johnathan Scott, USC School of Medicine, Los Angeles, CA 90032

Murine lymphoid cell system is a useful model for studying differentiation. We have derived a hematopoietic cell line from a hybrid embryo of AKR X wild mouse. Finely minced tissues from a 10 days' gestation "embryo" resulted in a rapidly growing culture of adhering cells that appeared to give rise "de novo" to rounded lymphoid cells. These hematopoietic cells have been in culture for about 10 months without any signs of deterioration. The cells proliferated rapidly with a doubling time of about 20 hours and spontaneously released murine leukemia viruses (MuLV). By end-point dilution techniques, we separated two distinct retroviruses; one similar to the prototype AKR ecotropic MuLV and another related to amphotropic MuLV of wild mouse. Direct immunofluorescence staining of the suspension cells with polyvalent and monovalent antisera against IgA and kappa light chain showed strong cell surface staining on about 80% cells. Low levels of staining was also seen by antisera to IgM and IgD. However, these cells were totally negative for benzidine stain for hemoglobin and Thy 1.2 antigen or terminal deoxynucleotidyl transferase activity. Also, inoculation of these cultures in nude mice did not produce any tumor. Thus, these cultures appear to consist of a clonal population of lymphoblasts of B-cell origin. Since this is a continuous cell line these cells will provide a useful tool for a better understanding of gene regulation and differentiation.

0607 GENETIC RESISTANCE OF MACROPHAGE CULTURES TO MHV-A59, Marilyn S. Smith, Robert E. Click and Peter G.W. Plagemann, University of Minnesota, Minneapolis, MN 55455

We have previously shown (Abstr. Annu. Meet. Am. Soc. Microbiol. T191, 1980) that cultured peritoneal macrophages from SJL mice are resistant to productive infection by mouse hepatitis virus strain A59 (MHV-A59), whereas macrophages from 8 other mouse strains are fully susceptible. In the present study, we determined whether the mechanism(s) which underlies resistance was under genetic control. For these studies, classical genetic segregation analysis was done with progeny derived from SJL and various H-2 congenic strains (congenic with C57BL/10Sn). The results indicate that resistance of macrophages derived from progeny of B10.S($H\text{-}2^s$) and SJL ($H\text{-}2^s$) to MHV-A59 replication was controlled by two independently segregating loci (at least one of which was expressed in a recessive manner). In contrast, resistance to replication of macrophages of progeny of B10.D2 ($H\text{-}2^d$) and SJL, B10.K ($H\text{-}2^k$) and SJL, and B10 ($H\text{-}2^b$) and SJL was controlled by an apparently single recessive gene. Since all of the B10 mice are genetically identical except at H-2, the difference in the apparent number of loci controlling resistance must be controlled in some manner by an H-2 associated gene(s) in addition to the two non-H-2 loci defined by SJL and B10.S progeny. Linkage of one of these two non-H-2 loci with c and p indicated that one locus is located on chromosome 7. In addition, since the map distance from c was similar irrespective of the B10 congenic parents, this locus is the single recessive segregating locus defined by SJL and B10, B10.K and B10.D2 progeny. We are attempting to map the H-2 locus with H-2 recombinant B10 mice.

0608 TRANSFORMATION-DEPENDENT DECREASE OF α ACTIN IN CULTURED CHICKEN EMBRYO FIBROBLASTS. D.J. Brown, D.P. Witt and J.A. Gordon, University of Colorado Health Sciences Center, Denver, Colorado 80262

In vitro tissue cultures of normal chicken embryo fibroblasts (CEF) obtained from 10 day old embryos, display three forms of actin, α, β and γ, as resolved by two-dimensional gel electrophoresis. The level of α actin is significantly reduced relative to β and γ actin after infection and transformation by Rous sarcoma virus (RSV). The reduction of α actin in transformed RSV-CEF is apparently due to a decreased synthetic rate as measured by ^{35}S-methionine pulse-label experiments. The possibility that the infection with RSV selects for a particular cell type within the culture that lacks α actin was examined by use of a temperature-sensitive mutant, tsNY68. We observed that at the permissive temperature, the transformed CEF display the reduced α actin levels. After a shift to the non-permissive temperature, α actin synthesis increases and reaches normal levels within 24 hours. These results eliminate the possibility of cell selection. We conclude that the decrease in α actin levels is a result of transformation and not a productive viral infection. We also note that the previously observed alteration in tropomyosin in CEF upon transformation correlates with the time course of change in α actin levels in tsNY68 temperature shift experiments. These selective decreases in two cytoskeletal proteins may account for some of the well recognized alterations in transformed cellular morphology.

0609 QUANTITATION OF CALCIUM REQUIREMENT FOR HUMAN PRECURSOR CELL PROLIFERATION AND MATURATION AND FOR LYMPHOCYTE BLASTOGENESIS, Camille N.Abboud, Andrew H. Lichtman, James K. Brennan, George B. Segel and Marshall A. Lichtman, University of Rochester School of Medicine, Rochester, New York, 14642.

We have studied calcium requirement for cell proliferation in two in vitro systems.Firstly, we estimated calcium requirements for human erythroid and granulocytic progenitors grown in a cal cium and magnesium deficient semi-solid matrix.In reconstituted cultures no growth was seen prior to the addition of calcium or magnesium.Small amount of either cation restored growth in the presence of saturating amounts (1mM) of the alternative cation.The fifty per cent (ID-50) inhibition was determined by EGTA titrations in the presence of 1mM magnesium chloride. This end-point was also correlated with calcium titrations in the presence of 1mM magnesium and just maximally inhibitory doses of EGTA (.175 mM).This indicated that at 0.025mM calcium (in excess of EGTA-binding capacity) 50% of normal growth occured, however higher calcium levels were required for optimal hemoglobinization of erythroid BFU-E. Secondly, in order to be able to measure calcium requirements for cell proliferation we developped a serum-free system for lymphocyte transformation after lectin activation. Calcium requirements for optimal tritiated thymidine incorporation into DNA at 72 hours, were determined by EGTA titrations and calcium titrations in the presence of 1mM magnesium chloride. Finally, ionized calcium was measured with a calcium electrode. As little as 40-50 micromoles of calcium were sufficient to restore lymphocyte proliferation by 50%. This value is also close to the one estimated for progenitor growth. This system can be adapted to the quantitation of calcium regulation of differentiation in other cell lines such as the K562 line.

0610 CLONING OF FUNCTIONAL GENES FOR SURFACE ANTIGENS INVOLVED IN HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA, Clifford P. Stanners, Teresa Lam, John W. Chamberlain, Stephen S. Stewart and Gerald B. Price, Ontario Cancer Institute, 500 Sherbourne St., Toronto, Ontario, Canada M4X 1K9.

A cell-cell hybrid between a CHO cell line and human lymphoblasts from a patient with B-cell chronic lymphocytic leukemia (CLL) was shown to produce a surface antigen detectable by a monoclonal antibody. This surface antigen can be detected on a fraction of chronic lymphocytic leukemia cells but not on normal human lymphocytes. Two different clones, HSAG-1 and HSAG-2, capable of transforming mouse cells to produce the surface antigen were isolated from a gene library of the hybrid cells by identification of small portions of the library containing functional genes followed by detection of clones with human-specific reiterated sequences. Both clones also contained CHO-specific reiterated sequences and are therefore human-CHO recombinants. HSAG-1 and HSAG-2 have related but not identical antigen-producing nucleotide sequences; HSAG-1 was found to be about 10-fold more potent than HSAG-2 in producing antigenic transformation. HSAG-1 could also produce inefficient morphological transformation (extreme cell rounding) of L cells. HSAG-like genes were present in the hybrid cell genome at a functional copy number of 100 to 1000. Hybridization analysis using portions of HSAG-1 not containing the human or CHO-specific reiterated sequences yielded molecular copy numbers of at least 1000 in both the hybrid cell and human CLL cell genome. We have therefore isolated two examples of a very large (middle repetitive) family of genes which have a defined function correlated with certain forms of human leukemia.

0611 BIOCHEMICAL AND SEROLOGICAL APPROACHES TO MOLECULAR CHARACTERIZATION OF INTERLEUKIN-2
D. Mochizuki and S. Gillis, Hutchinson Cancer Center, Seattle, WA 98104

A number of factors appear to regulate the growth and differentiation of hemopoietic cells. We feel that a thorough assessment of mode of action and potential therapeutic value is dependent on biochemical and molecular characterization. Utilizing serum-free culture supernates from both mitogen stimulated human PBL and those prepared by ligand stimulation of Jurkat FHCRC human leukemia cells, we have purified human IL-2 to apparent molecular homogeneity. Efforts have been helped by the generation of a monoclonal antibody directed against a determinant on human IL-2. Reaction of ^{125}I-purified human IL-2 with anti IL-2 IgG and lyophilized Staphyloccus aureus (Igsorb) resulted in precipitation of a 13,000 mw protein as visualized by autoradiography of electrophoresed precipitates. Immune precipitation of radiolabeled IL-2 (produced biosynthetically by exposure of Jurkat cells to ^{35}S-methionine/^{3}H-leucine prior to ligand induction) was dependent on the concentration of antibody used and was inhibited by inclusion of cold IL-2 in the primary RIA reaction. Precipitation was also inhibited by addition of the rabbit reticulocyte translation product of Jurkat derived human IL-2 mRNA. Jurkat mRNA reticulocyte translation product retained biological activity in a modified IL-2 assay for T cell line proliferation. Finally, immune precipitation of ^{35}S-methionine labeled Jurkat mRNA translation product resulted in precipitation of approximately 2% of the total radioactivity. Electrophoresis and 72 hour autoradiography revealed precipitation of proteins suggesting the involvement of post-translational processing for evolution of biologically active human IL-2.

0612 IN VITRO GROWTH OF BONE MARROW DERIVED T LYMPHOCYTES, P. Conlon, C. S. Henney, and S. Gillis. Program in Basic Immunology, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The importance of regulatory molecules and cell-derived factors in the development and differentiation of the immune system has long been appreciated. Numerous reports by this laboratory and others have demonstrated that the long-term growth of T-lymphocytes can be achieved with highly purified lymphokines derived from human, rat, and mouse cell conditioned medium. In an attempt to better comprehend the differentiation processes involved in the maturation of T cells, we attempted to maintain in vitro bone marrow derived cells in the presence of various factors. As a source of conditioned medium, we have used culture supernatants from the growth of the murine myelomonocytic leukemic cell line, WeHi-3. We observe the long term growth (6 months) of a murine lymphocyte population from the bone marrow. By FACS analysis, these cells were thy 1.2^{+}, Lyt 1.2^{-}, Lyt 2.2^{-}, and $H\text{-}2^{+}$. Furthermore, although initially dependent on WeHi-3 conditioned medium, the bone marrow cells no longer required its presence for growth. Once independent, such cells produce high levels of IL-2 in the absence of exogenous antigens. Such cells have been cloned by limiting dilution and all clones produce IL-2 to varying degrees. The factor(s) responsible for this in vitro growth and maturation are currently under investigation.

0613 CHARACTERIZATION OF THE (Na,K)-ATPase IN MURINE ERYTHROLEUKEMIA CELL MEMBRANES,
Li-An Yeh, Leigh English, Leona Ling and Lewis Cantley, Harvard University, Dept. of Biochemistry and Molecular Biology, Cambridge, MA 02138

Friend virus transformed murine erythroleukemia (MEL) cells differentiate into hemoglobin producing red cell precursors when treated with a variety of inducers, including the specific (Na,K)-ATPase inhibitor, ouabain (A. Bernstein, V. Hunt, V. Crichley, and T.W. Mak, 1976, Cell 9, 375-381). The plasma membrane from uninduced cells was purified and characterized. Approximately 30% of the ATPase activity in these membranes is Na^{+} dependent and ouabain inhibitable and 5% is Ca^{2+} dependent. Antibodies prepared against the α subunit of dog kidney (Na,K)-ATPase precipitate a ~100,000 dalton protein of MEL cell plasma membranes. This protein is phosphorylated by an endogenous plasma membrane bound kinase. The plasma membrane kinase will also phosphorylate exogenously added dog kidney on both the α and β subunits. The effect of various inducing agents on the plasma membrane kinase activity will be discussed.

0614 DETECTION OF SV40 LARGE T-ANTIGEN AND 53K CELLULAR PROTEIN ON THE SURFACE OF SV40-TRANSFORMED MOUSE CELLS USING MONOCLONAL ANTIBODIES, Myriam Santos and Janet S. Butel, Baylor College of Medicine, Houston, TX 77030

Altered cell surface properties of SV40-transformed cells might be related to the presence of SV40 large T-antigen (T-ag) on the surface of the cells or to an interaction between T-ag and some specific cellular protein. Previous studies of surface-associated T-ag employed sera from SV40 tumor-bearing hamsters or antisera prepared against purified T-ag. We have now utilized a series of monoclonal antibodies to determine what portions of the T-ag molecule are exposed on the surface of SV40-transformed cells. The monoclonal antibodies (from E. Harlow and E. Gurney) were directed against different antigenic sites on the T-ag molecule, including sites on the amino and carboxy termini. We have previously shown that surface T-ag can be specifically precipitated during the external reaction of a differential immunoprecipitation technique. Cultures of SV40-transformed mouse cells were surface-iodinated and were then incubated with monoclonal antibodies to T-ag, followed by a second incubation with goat anti-mouse IgG serum. Cells were disrupted, the extracts clarified, the immune complexes absorbed with protein A-containing *Staph. aureus*, and the precipitated proteins analyzed by polyacrylamide gel electrophoresis. Iodinated T-ag was precipitated by all the different monoclonal antibodies utilized. Similar results were also obtained with a radioimmune assay using ^{125}I-labeled protein A to bind to antigen-antibody complexes on the cell surface. These results suggest that both the amino and carboxy termini of the T-ag molecule are exposed on the transformed cell surface. The precise nature of the association of T-ag with the cell membrane remains to be determined. Most of the immunoprecipitates formed by monoclonal antibodies to T-ag also contained 53K cellular protein, providing further support for the existence of a complex of large T-ag and 53K on the surface of SV40-transformed mouse cells.

0615 MURINE LEUKEMIA VIRUSES IN DBA/2 MICE, Bjørn Andersen Nexø and Kay Ulrich, The Fibiger Laboratory, Copenhagen Ø, Denmark.

Mice of the DBA/2 strain have two unusual properties in relation to leukemia viruses: (1) The mice display an exceptionally high level of MuLV-related proteins in a variety of tissues, including kidney, epididymis, uterus, and thymus; The proteins appear in many instances located in epithelial cells. (2) Mice of this strain are susceptible to infection with many murine leukemia viruses, but resistant to their leukemogenic action.
We have characterized in detail the protein structure of viruses isolated from these mice, and have demonstrated the presence of three radically different envelope glycoproteins and two rather different core protein structures among theviruses. The relation of these proteins to the tissue-specific, constitutively expressed MuLV-related proteins, and their importance to the biology of DBA/2 mice is currently being investigated.

0616 MOUSE GENES ACTIVATED IN SV40-TRANSFORMED CELLS. Peter W.J. Rigby, Paul M. Brickell, Karl-Heinz Westphal and Michael R.D. Scott, Cancer Research Campaign, Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College of Science and Technology, London SW7 2AZ, England.

We have constructed cDNA libraries from normal Balb/c 3T3 cells and from a SV40-transformed derivative, SV3T3 C138. Preparative hybridization between C138 cDNA and the 3T3 library was used to prepare a probe enriched for transformed-cell-specific sequences and we thus isolated from the C138 library clones which correspond to RNAs present at elevated levels in C138. These clones can be divided into five families, three of which are related to each other in a complex fashion. Two of the families hybridize to single mRNA species which are ten- to fifty-fold more abundant in C138 than in 3T3. The other families contain sequences repeated within the RNA population and hybridize to highly abundant non-regulated RNAs as well as to much less abundant RNAs which are C138-specific. One family contains a sequence highly repeated in the mouse genome while another contains only unique sequences. The three related families all hybridize to restriction fragments repeated several hundred times. We are presently seeking to identify the products of these genes and to characterize them in more detail. We are also screening mouse cells transformed by other agents, both viral and chemical, to see if elevated expression of these genes is a common feature of transformation.

0617 EXPRESSION OF CELL DIFFERENTIATION AND CELL TRANSFORMATION BY EMBRYONIC CHONDROBLAST PRECURSOR CELLS INFECTED WITH ROUS SARCOMA VIRUS, David Boettiger, University of Pennsylvania, Philadelphia, PA 19104

Mesenchymal cells were taken from stage 22 chicken embryo limb buds, dissociated with trypsin to produce a single cell suspension, infected with 5-10 FFU/cell with a ts mutant of Rous sarcoma virus and plated under culture conditions which allow the differentiation of chondroblasts, fibroblasts and myoblasts. The experiments described have concentrated on the chondroblast differentiation. The process was followed morphologically, histochemically using a staining procedure specific for sulfated proteoglycans and biochemically by analysis of expression of chondroblast-specific sulfated proteoglycan.

Under our standard culture conditions chondroblast markers could first be detected on day 2 or 3 (depending on incubation temperature) and exhibited continuous quantitative increase in sulfated-proteoglycan of the chondroblast type and successive stages of morphological development. Cells infected with ts-RSV and incubated either at 36° or 41° appear to exhibit an accelerated chondroblast differentiation. This process continues in infected cells at 41°, but following the initial expression of chondrogenesis in infected cultures at 36°, there is a suppression of the chondroblast phenotype as the cells transform. The transformed phenotype is fully temperature sensitive and may be reversed by a shift to 41°. These results suggest that the transforming effects of Rous sarcoma virus are not expressed in presumptive chondroblasts but require the expression of cell products associated with the more mature chondroblasts.

0618 5-AZACYTIDINE AND 5-AZA-2'-DEOXYCYTIDINE AS INDUCERS OF DIFFERENTIATION AND INHIBITORS OF DNA METHYLATION IN FRIEND ERYTHROLEUKEMIA CELLS. Judith K. Christman, Francine Creusot and George Acs, Mount Sinai School of Medicine, New York, N. Y., 10029.

Substantial evidence indicates that enzymatic methylation of cytosine (C) residues in DNA may influence the expression of genes in vertebrate cells. The antileukemic drugs, 5-azacytidine (5-aza-CR) and 5-aza-2'deoxycytidine (5-aza-CdR) act specifically as inhibitors of methylation of C residues in DNA and RNA of pro- and eukaryotic cells. Both of these analogs act as weak inducers of differentiation of Friend erythroleukemia cells. 24 h exposure to 2 μM 5-aza-CR or 0.2 μM 5-aza-CdR induces differentiation of 10-15% of cells and enhances their ability to differentiate in the presence of suboptimal levels of Me_2SO. DNA synthesized during the period of analog treatment is markedly hypomethylated, having a 5mC/C ratio 40% lower than DNA from untreated cells. However, within 24-48 h after terminating exposure, gross hypomethylation of DNA in the treated cells is no longer detectable. These results are directly related to the effect of 5-aza-CR and 5-aza-CdR on the activity of DNA methyltransferase. Depending on the concentration of analogs employed, virtually complete loss of DNA methyltransferase occurs within 4-8 h. Within 24-48 h after the cells are washed free of the analogs, normal levels of DNA methyltransferase are restored. Hydroxyurea blocks the effect of 5-aza-CR and 5-aza-CdR on DNA methyltransferase activity, indicating that they must be incorporated into DNA to act as inhibitors. In vitro, 5-aza-C substituted DNA forms a complex with DNA methyltransferase which is stable in the presence of 1M NaCl and 0.6% sarkosyl. This tight binding may explain our observation that substitution of as few as 0.3% of C residues in DNA with 5-aza-C is sufficient to cause complete inactivation of the enzyme in vivo.

0619 SPECIFICALLY DRUG-RESISTANT, CULTURED HUMAN MYELOID LEUKEMIA (HL60) CELLS CONTAIN DOUBLE MINUTE CHROMOSOMES. Robert E. Gallagher, Anna C. Ferrari, and Joseph R. Testa, Baltimore Cancer Research Center, 655 W. Baltimore Street, Baltimore, MD 21201

Drug-resistant sublines of HL60 cells were developed by separately exposing parental cultures to gradually increasing doses of three chemical inducers of granulocyte differentiation–dimethylsulfoxide (DMSO), retinoic acid (RA), and 6-thioguanine (6TG). For the DMSO- and RA-resistant sublines, the acquisition of the ability to "grow-through" concentrations of drug which produced differentiative extinction of the parental line (1.2% DMSO; 10^{-6}M RA) was associated with the loss of differentiation in response to that particular agent. For the 6TG-resistant subline, resistance to the cytotoxic effect of 6TG was acquired at lower levels of cellular resistance (0.5 to 5 μg/ml 6TG) than the loss of the differentiative response (20 to 50 μg/ml). The latter appeared cytologically complete for 6TG and hypoxanthine, partial for DMSO, and absent for RA. In the RA- and 6TG-resistant sublines, numerous double minute chromosomes (dmc) were observed. In the 6TG-resistant subline, which has been most thoroughly analyzed cytogenetically, the number of dmc increased up to a cellular resistance level of 20 μg/ml and then disappeared in cells adapted to 50 μg/ml. In other culture systems, dmc have been associated with experimentally-amplified genes specifically related to the selective agent. We are investigating the possibility, suggested by the biological specificities of the variant sublines, that the HL60 cell culture line may have generated amplified copies of different genes during the development of resistance to agents which affect growth and differentiation.

0620 ISOLATION AND CHARACTERIZATION OF MURINE HEMATOPIETIC PROGENITOR CELLS USING MONOCLONAL ANTIBODIES, Joan W. Berman and Ross S. Basch, NYU Medical Center, New York, New York, 10016

Monoclonal antibodies with specificity for antigens found on murine bone marrow cells have been prepared. We have used these antibodies to isolate highly enriched populations of progenitor cells and have studied the behavior of these cells after tranplantation and in culture. Several specificities expressed on multipotential stem cells (CFU-s) have been defined. One of these persists during macrophage-granulocyte differentiation, while another is retained on some cells of the T-cell lineage. Two others react only with immature cells.

The multipotential stem cell pool identified by these reagents is not homogeneous and although all of the antibodies are cytotoxic, none is capable of completely eliminating CFU-s. Mixing experiments have identified two complementary groups of antisera which together eliminate virtually all of the progenitors of macroscopic spleen colonies.

Cell sorting, using a fluorescence-activated cell sorter has allowed us to separate cells reactive with each of the antisera. We have thus been able to study the ability of these cells to achieve engraftment, the effect of "helper" cells and/or factors on this process and the subsequent differentiation in the irradiated host. We have also begun to analyze the conditions required for the growth of these cells in culture. (Supported by Grant ROI CA 24472 of the NIH, USPHS.)

0621 RECRUITEMENT OF A CFU-S-LIKE POPULATION BY MURINE LEUKEMIA VIRUS (RadLV): THE ESTABLISHMENT OF CONTINUOUS CFU-S PRODUCER CELL LINES, Paul Jolicoeur and Perin Sankar-Mistry, Clinical Research Institute of Montreal, Montreal, Canada, H2W-1R7

Two strains of murine leukemia virus (MuLV) isolated from X-irradiated-induced thymomas of the C57BL/Ka mouse were used to infect C57BL/Ka spleen cells in vitro . Continuous cell lines resulting from such infections have been established and are being maintained in culture for over 11 months. The adherent cell layer of these cultures produces CFU-S-like cells able to give rise to granulocytic, erythrocytic and undifferentiated colonies in spleen of irradiated mice within 10 days. The cells released in the supernatant of these cultures are morphologically heterogenous: immature granulocytes, monocytes, reticulocytes and lymphocyte-like cells have been observed. The cultures also harbor CFU-C cells. A B-tropic fibrotropic MuLV is released by these cultures. Unlike the parental viral strains used to infect the spleen cells, the ensuing MuLV has not as yet (10 months) proved to be leukemogenic. Also the cultured cells do not induce tumors when injected intra-peritoneally into C57BL/Ka mice. It thus appears that these MuLV strains have the ability to promote the growth of a CFU-S-like cell in vitro.

0622 EXPRESSION OF CLONED GLOBIN GENES AFTER TRANSFER TO MOUSE ERYTHROLEUKEMIA CELLS, Pamela Mellon*, Moses Chao+, Barbara Wold+, Tom Maniatis* and Richard Axel+, Harvard University, Cambridge, MA 02138 * and Columbia University, New York, NY 10032 +

We have investigated the expression of cloned hemoglobin genes which have been introduced into mouse erythroleukemia cells by DNA-mediated gene transfer. The human α- and β-globin genes as well as a mouse 5' βmajor-human 3' β globin hybrid gene have each been introduced by cotransformation into MEL cells deficient in either Tk or Aprt activity. Stable transformants were isolated and shown to contain from 1-100 copies of the exogenous globin genes. Upon induction of the human α-and β-containing MEL cell transformants with DMSO or HMBA, we have not yet observed any human MRNA induction, even though the endogenous mouse β gene is induced. A varying constituitive level of synthesis of human globin mRNA is observed in these lines. However, the mouse-human hybrid β gene will coinduce in MEL cells to levels of 2000 copies of mRNA per cell, a value closely approximating the endogenous mouse globin gene. These data imply that the information required for the inductive process may be species specific and resides within the approximately 1.3 kb of 5'-flanking mouse DNA present on the hybrid cloned β-globin gene. We are presently using in vitro-truncated DNA clones with less mouse 5'-flanking DNA to define more precisely those DNA sequences which are required for the induction of gene expression during MEL cell differentiation.

0623 CHARACTERIZATION OF HEMOPOIETIC PRECURSOR CELLS IN MURINE FETAL LIVER AND BONE MARROW. Philip Zoltick and Joan Abbott, Sloan-Kettering Institute, New York,NY 10021

Two recently discovered murine allo-antigens, Ly10.1 and Ly18.1, detected by monoclonal anti bodies, are present on both T and B lymphocytes, as well as on unidentifed bone marrow cells Using fluorescence, cytotoxicity and rosetting assays, we have shown that 90% of 15-16 day fetal liver cells and 50-70% of neonatal and adult bone marrow cells are Ly10.1 positive. Ly18.1 is found on 8% of 15-16 day fetal liver cells and 25-30% of neonatal and 35-38% of adult bone marrow cells. It is deduced from histologic examinations of cultured Ly10.1 depleted bone marrow and fetal liver cells that Ly10.1 is expressed on precursor cells which give rise to granulocytes, erythrocytes and lymphocytes. Ly10.1 is not on more mature granulocytes or erythrocytes. Double labeling studies have shown that 90% of the few sIgM positive cells in bone marrow are also Ly10.1 positive, but only 22% of the Ly10.1 positive cells are sIgM positive. Two murine precursor cell lines have been found positive for Ly10.1, the pre-B cell line 70Z/3 and 416B cell line which generates megakaryocyte and granulocyte progeny. Depletion of Ly10.1 positive cells from adult bone marrow results in 50-80% inhibition of M-CFC but no change in number of CFU-S compared to controls. The fact that CFU-S are not enriched following depletion suggests that some CFU-S in bone marrow are Ly-10.1 positive. Ly18.1 depletion of adult bone marrow fails to inhibit either M-CFC or CFU-S. These results suggest that myeloid and lymphoid progenitors express the Ly10.1 antigen.

0624 MOLECULAR CLONING OF HUMAN CELLULAR SEQUENCES HOMOLOGOUS TO THE TRANSFORMING GENES OF ROUS SARCOMA VIRUS (SRC), AND AVIAN ERYTHROBLASTOSIS VIRUS (ERB). Donald J. Fujita, Janet Radul, Joseph Baar, Martin Evers, Steven Anderson, Carol Gibbs*, and Hsing-Jien Kung*, Cancer Research Laboratory and Dept. of Biochemistry, Univ. of Western Ontario, London, Canada, and Dept. of Biochemistry*, Michigan State Univ., East Lansing, Michigan.

Sequences homologous to transforming genes, or "oncogenes", of RNA tumor viruses are present in the genomes of all vertebrate species. Such sequences are presumed to have important functional roles in normal cells, and may be involved in at least some types of neoplastic disorders.

We have isolated several molecular clones containing human DNA sequences homologous either to the Rous sarcoma virus transforming gene (src), or to transforming gene sequences of avian erythroblastosis virus (erb), utilizing lambda Charon phage human genomic libraries provided by E. Fritsch (Fritsch et al, Cell 19, 959 (1980)), and a modification of the plaque screening protocol of Benton and Davis. Our initial DNA endonuclease mapping studies performed on DNAs purified from recombinant clones suggest that human cellular src-related sequences (c-src) are dispersed over a distance of at least 7-10 kilobase pairs. This suggests that several intervening sequences, or introns, are present. Results of DNA restriction and EM-heteroduplex mapping experiments will be presented.

0625 CELLULAR PROTEINS CROSSREACTING WITH POLYOMA VIRUS MEDIUM T ANTIGEN, Gernot Walter and Hermann Werchau, University of Freiburg and University of Bochum, F.R.G.

Antiserum against the synthetic peptide Lys·Arg·Ser·Arg·His·Phe, corresponding to the carboxyterminus of polyoma virus medium T antigen (mT), immunoprecipitates not only mT but also two cellular proteins with molecular weights of 30000 and 26000. They are present in polyoma virus infected and uninfected mouse cells (1). When infected or uninfected cell extracts are first treated with the SH-group reagent N-ethylmaleimide (NEM) then a third protein with a molecular weight of 36000 is recognized by the anti-peptide serum. Therefore, this protein shares an antigenic determinant with mT that is normally buried inside the protein or covered up by another protein or cellular structure. The two-dimensional tryptic fingerprints of the 36K protein and of mT are not apparently related to each other. The 36K protein is a nuclear protein, which is not phosphorylated. The problem of crossreactivities of anti-peptide sera and of monoclonal antibodies will be discussed.

(1) G. Walter, M.A. Hutchinson, T. Hunter, and W. Eckhart; PNAS 78, 4882 (1981).

0626 TRANSFORMATION OF HUMAN EMBRYONIC KIDNEY CELLS USING A MUTANT OF SV40 AND THEIR USE AS HOSTS FOR HUMAN PAPOVAVIRUS MULTIPLICATION, Eugene O. Major, National Institute of Neurological and Communicative Disorders and Stroke, NIH, Bethesda, MD 20205.

Human embryonic kidney cells were transformed at their third passage by an origin defective mutant of SV40 (Y. Gluzman, et al., Proc. Nat. Acad. Sci, 77, 1980). The presence of both large and small T proteins could be demonstrated following immunoprecipitation and resolution on SDS PAGE gels. Fluorescent antibody assays using anti-SV40 tumor serum revealed that independent clones of transformed cells varied in the number of cells producing T protein. One clone demonstrated nearly 100% positive expression of T protein which could be precipitated using a monoclonal antibody against the SV40 T protein. The frequency of transformation could be augmented if the SV40 mutant DNA was cotransfected with a plasmid DNA containing the replication origin of BK virus. The BK fragment DNA, however, could not be shown to independently replicate in HEK transformed cells, even though the SV40 T protein was present. BK and JC virus form I DNA, both human papovaviruses which demonstrate narrow host ranges for lytic growth, were transfected into several clones of transformed HEK cells. Results of these experiments will be presented.

0627 EXPRESSION OF HTLV ANTIGENS IN A JAPANESE ADULT T-CELL LEUKEMIA (ATL) LINE AND ANTIBODIES TO THEM IN ATL SERA, V.S. Kalyanaraman, M. Popovic, M. Robert-Guroff, M.G. Sarngadharan, and R.C. Gallo. Litton Bionetics, Inc., and Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, MD 20205

A unique animal retrovirus designated HTLV, was isolated from cultured T-cells of some patients in the U.S. with leukemias and lymphomas involving relatively mature T-cells. This virus was not endogenous to humans and is acquired by exogenous infection. A cell line derived from a Japanese ATL patient was analyzed for the expression of components of HTLV. By competition radioimmunoassay and by indirect immunofluorescence assay these cells were found to express low levels of HTLV core proteins p24 and p19, respectively. Upon induction with IdUrd, the concentration of p24 and the proportion of p19-positive cells increased approximately 8-fold. This finding parallels the results of extensive survey of Japanese ATL sera that indicate the presence of antibodies to HTLV antigens in >90% of the sera examined. A strong association of HTLV with T-cell malignancies in parts of Japan is indicated by the above data.

0628 IN VITRO GROWTH AND DIFFERENTIATION OF HUMAN CORD BLOOD MYELOID AND MONOCYTOID CELLS FOLLOWING EXPOSURE TO PRIMATE TYPE-C RETROVIRUSES, P.D. Markham, S.Z. Salahuddin and R.C. Gallo, Lab. of Tumor Cell Biol., NCI, Bethesda, MD 20205

Long-term suspension cultures of myeloid cells and monocytes are readily established from human cord blood leukocytes by cultivation of immature cells prepared on Percoll gradients in media supplemented with specific hormones, i.e., hydrocortisone and vitamin D (HD-RPMI). These cultures, which develop in ∿50% of the samples tested, are initially composed predominantly of immature myeloid cells and a low level of monocytes, and undergo gradual maturation until they terminate as mature neutrophils and monocyte-macrophages. Since several animal retroviruses are able to infect and transform hematopoietic cells and others exhibit possible blastogenic or mitogenic effects, an attempt was made to further extend cord blood leukocyte in vitro growth by infection of fresh or cultured immature cells with one of several primate retroviruses, e.g., simian sarcoma virus (SSV) and gibbon ape leukemia virus (GALV), in combination with HD-RPMI.

The introduction of these viruses into preparations of cord blood leukocytes has, so far, not afforded additional growth capabilities to cultures in the presence of HD-RPMI. However, virus infection was found to be efficient in replacing the need for the hormone supplemented media in initiating and supporting the long-term myeloid and monocytoid cell growth in ∿20% of 50 cord blood samples tested. The recent human virus isolate, HTLV, was also tested and found to be able to replace HD-RPMI in long-term leukocyte replication. HTLV therefore behaves similarly to two other primate type-C viruses in its ability to stimulate and support long-term in vitro leukocyte growth.

0629 FACTORS DETERMINING THE SUSCEPTIBILITY OF NIH SWISS MICE TO ERYTHROLEUKEMIA INDUCED BY FRIEND MURINE LEUKEMIA VIRUS, Sandra Ruscetti, John Feild, Lenora Davis and Allen Oliff*, National Cancer Institute, Bethesda Md. 20205 and *Memorial Sloan Kettering Cancer Center, New York, New York 10021

Helper-independent virus from a molecularly cloned stock of Friend murine leukemia virus induces an erythroproliferative disease characterized by splenomegaly and severe anemia in newborn mice of certain strains, such as NIH Swiss. With age, NIH Swiss mice become increasingly resistant to this F-MuLV-induced disease. Resistance is not correlated with the replication of the input Friend MuLV but is correlated with the replication of Friend mink cell focus-inducing (MCF) viruses in the spleens of infected mice. Treatment of adult NIH Swiss mice with x-irradiation, phenylhydrazine or silica before injection of F-MuLV increases the replication of MCF viruses in the spleens of these mice and renders them significantly more susceptible to erythroleukemia. A humoral immune response to MCF gp70 appears to play a role in this resistance, for antibodies capable of precipitating MCF gp70 can be detected in the sera of mice infected with F-MuLV at 6 weeks of age but not in mice infected as newborns or as adults after irradiation. Phenylhydrazine-treated adults, however, had serum antibody levels to MCF gp70 equivalent to untreated adults, indicating that additional factors other than an anti-viral immune response are involved in this age-associated resistance.

These results suggest that the resistance of adult NIH Swiss mice to erythroleukemia induced by F-MuLV is due to the lack of replication of Friend MCF viruses in these mice because because of (1) a good immune response to the virus; (2) the lack of a suitable number of susceptible target cells; or (3) both.

0630 FRIEND VIRUS-INDUCED TUMOR CELL LINE IN MICE RESISTANT AT THE FV-2 LOCUS, Rita Anand* Mark Dizik, Alan Berkower, Roy Geib and Frank Lilly, Albert Einstein College of Medicine, Bronx, NY 10461

Friend virus (FV)-induced erythroleukemia in mice is controlled by the FV-2 gene carrying two alleles, FV-2^S for susceptibility and FV-2^r for resistance. Erythroleukemia cell lines from the FV-induced tumors in FV-2^S mice have been produced and characterized earlier. In an effort to understand the mechanism of the FV-2^r gene we produced a tumor in D2.FV-2^r mice by multiple injections of FV followed by the subcutaneous transplantation of the infected spleens in syngeneic hosts. The tumor was adapted to grow in cell culture. The virus produced from the cells is NB-tropic, XC positive and causes spleen foci and polycythemic erythroleukemia in FV-2^S mice. The cells are tumorigenic and induce colony formation in both FV-2^S and FV-2^r mice. Intracellular expression of Friend-murine leukemia virus (FMuLV) and spleen focus forming virus (SFFV) was analyzed by the immunoprecipitation of viral proteins and by hybridization of cellular RNA with SFFV-specific ^{C}DNAs. Virus expression was comparable to the expression in other well studied erythroleukemic cell lines produced in FV-2^S mice. However, some qualitative and quantitative change in viral proteins and RNA was observed in this cell line. Since the cells were morphologically erythroid and were DMSO-inducible it can be inferred that the observed changes in the FV complex might be contributing to its ability to cause erythroleukemia in resistant mice.

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0631 ERYTHROPOIETIN-INDUCED ERYTHROPOIESIS IN LONG-TERM BONE MARROW SUSPENSION CULTURES: INHIBITION BY HUMORAL FACTOR(S) Christine Eastment and Francis Ruscetti,Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Md. 20205

Unlike murine bone marrow cultures, hamster long-term marrow cultures do not require an adherent layer for maintenance of stem cell proliferation. While the cultures are largely myeloid and macrophage, a number of megakaryocytes and "blasts" are observed. No hemoglobinized cells as judged by benzidine staining (BPC) are seen in these cultures. Addition of erythropoietin (epo) to 2-5 month old cultures results in the appearance of BPC within 4 days and the generation of mature normoblasts and non-nucleated erythroid elements within 7 days. If these suspension cells plus epo are incubated in the presence of an autologous parental adherent layer, erythropoiesis is completely inhibited. Prior stimulation of these cells with epo for 24 hours before addition to the adherent layer still results in inhibition. These suspension cells can be removed from the adherent layer after 7 days and stimulated to go through erythropoiesis. Conditioned medium (CM) from these adherent cultures also produces varying amount of inhibition(50-100%) of erythropoiesis suggesting that a humoral factor is at least partly responsible. Addition of CM to thesesuspension cells in semi-solid media stimulated by either epo or colony-stimulating factor causes no change in the number of CFU-C while the number of BFU-E is reduced 25-100% suggesting a specificity for erythroid development. Whether this factor is related to a recently reported (Cell 26:233,1981) one which inhibits DNA synthesis in murine BFU-E is not known. The factor present in hamster adherent cultures seems specific for erythroid cells, non-toxic to BFU-E and has reversibile effects suggesting that it may be physiologically important.

0632 MALIGNANT TRANSFORMATION OF PROADIPOCYTES: A SPECIFIC CELL CYCLE DEFECT IN CONTROL OF THE COUPLING OF GROWTH ARREST AND DIFFERENTIATION. J.J. Wille, Jr. and R.E. Scott, Section of Experimental Pathology, Mayo Clinic, Rochester, MN 55905.

Growth control of many stem cells *in vivo* is regulated by the coupling of growth arrest and differentiation. We have recently reported that this coupling process in proadipocytes is mediated at a state in the G_1 phase of the cell cycle, designated G_D, which is distinct from other G_1 growth arrest states induced by either growth factor deprivation (G_S) or nutrient deprivation (G_N). That is, prior to differentiation, proadipocytes must growth arrest at the G_D state. G_D arrested cells have: 1) a 2N content of DNA; 2) unique responsiveness to a mitogenic effect of methyl isobutyl xanthine; and 3) the ability to differentiate without DNA synthesis. We now describe the results of studies to determine whether malignant transformation of proadipocytes correlates best with defective cell cycle growth control mediated at G_D, G_S or G_N. BALB/c 3T3 T proadipocytes were transformed by the smooth surface tumorigenesis method. From these tumors, thirty-two noncloned and five cloned cell lines were derived. One hundred percent of these transformed proadipocyte cell lines lacked the capacity to G_D arrest and differentiate. By contrast, less than fifteen percent showed defects in G_1 growth control processes mediated at G_S or G_N. This strongly suggests that malignant transformation of proadipocytes is associated with or results from a selective defect in the coupling of growth arrest and differentiation. We are now performing studies to determine if transfection of proadipocytes with oncogenic human DNA from acute myelogenous leukemic cells also causes malignant transformation associated with defective coupling of growth arrest and differentiation. (Supported by NIH Grant CA 28240 to R.E.S.)

0633 THE COMPLETE AMINO ACID SEQUENCE OF GLYCOPROTEIN GP70 OF FRIEND-LEUKEMIA VIRUS, Robert Chen, Max-Planck-Institut für Biologie, Tuebingen, West Germany.

The complete amino acid sequence of glycoprotein GP70 of Friend leukemia virus (FLV) is presented. The sequence has been established by protein methods exclusively. The determinations were done in a micro scale and within a short time comparable to the DNA technique. A comparison of the amino acid sequences of GP70 of FLV and Mo-MuLV (Moloney muvine leukemia virus) as deduced from its DNA sequence is given.

0634 PRODUCTION OF COLONY STIMULATING ACTIVITY (CSA) BY MURINE BONE MARROW STROMA FOLLOWING IN VITRO IRRADIATION (XRT), Richard J. Gualtieri and Peter J. Quesenberry, University of Virginia, Charlottesville, Virginia 22908

It has been shown that lethally irradiated mice have elevated CSA levels. Radiation related gut injury with endotoxemia has been postulated as the underlying mechanism. Employing Dexter liquid cultures, we studied the effect of radiation on in vitro CSA production by murine (C57Bl) bone marrow adherent cells (stroma). Murine bone marrow cultures with established stroma were irradiated and stromal CSA production assayed by an in situ double-layer agar technique using the stroma as a feeder underlayer. CSA levels were expressed as the number of colonies stimulated per 19.4 cm^2 flask area. Stroma assayed immediately after XRT (week 1) showed dose-dependent CSA elevations as compared to non-irradiated flasks (Table). Persistent elevations of CSA were seen in irradiated flasks at week 2, while CSA levels from non-irradiated flasks fell. A dose dependent relationship was noted for colony size with huge colonies observed at the higher XRT doses. Colonies were primarily granulocyte/macrophage. However, acetylcholinesterase positive megakaryocyte colonies were also stimulated by stroma. These data indicate that 1) cultured marrow stroma produces CSA, 2) elevation of CSA can be induced by stromal XRT and 3) CSA is produced by a radioresistant stromal cell. Production of more than one stimulatory factor is suggested by detection of megakaryocyte colonies.

XRT Dose (rads)	Colonies (mean ± SEM) Week 1	Week 2
0	212 ± 34	91 ± 29
250	283 ± 62	275 ± 58
750	383 ± 28	452 ± 48
950	407 ± 35	479 ± 42

(blank underlayer = no growth)

0635 A NOVEL MECHANISM IN AVIAN LEUKEMOGENESIS AND LYMPHOMOGENESIS: ACTIVATION OF HOST ONCOGENES, C-ERB AND C-MYC, BY RETROVIRAL DNA INTEGRATIONS. Yuen-Kai Fung*, M.R. Noori-Daloii*, A. Fadly+, L.B. Crittenden+ and Hsing-Jien Kung*. *Department of Biochemistry, Michigan State University, East Lansing, Michigan; +USDA, SEA Regional Poultry Research Lab, East Lansing, Michigan.

Our labs have been engaged in studies to characterize the naturally occurring neoplasms induced in chicks by retroviruses. Lymphoid leukosis (LL) appears to be the most common one. Occasionally, erythroblastosis (Ery) and other neoplasms are also observed. Avian leukosis viruses (ALV) and chicken syncitial viruses (CSV, a member of the non-defective reticuloendotheliosis viruses) have been implicated as causative agents for these diseases. We (1,2) have previously reported that the initial molecular events involved in the LL induction by these two genetically unrelated viruses appear to be similar. In both cases, specific integration of proviral DNA near a host gene, c-myc, is observed. This implicates c-myc as a specific target gene for chicken B-lymphoma.

Recently, we have extended our analysis to the erythroblastoma (Ery) induced by ALV. We found that host gene c-erb is involved (c-erb is the cellular homolog to the oncogene of AEV, which induces acute erythroblastosis in chicken). The tumors induced by ALV appear to be of clonal origin (whereas the AEV induced ones are polyclonal). In 70% of the Ery tumors, new restriction fragments carrying erb sequences are found. Most of them carry sequences related to erb-B but not to erb-A. We have carried out extensive structural analyses on some of the altered erb fragments: in one of them, the linkage between erb_A and LTR can be demonstrated by molecular cloning. In the other the LTR appears to reside near the 5' end of erb_B locus. The expression of erb sequences in some of the tumors is elevated to a level comparable to that in AEV induced Ery. However, tumors which do not display enhanced erb expression are also observed.

0636 THE STRUCTURE AND ROLE OF INTEGRATED HEPATITIS B VIRUS SEQUENCES IN HEPATOCELLULAR CARCINOMA. Anne Dejean, Christian Bréchot, Pierre Tiollais and Simon Wain-Hobson, INSERM U.163, INSTITUT PASTEUR, 28 rue du Dr. Roux, 75724 PARIS CEDEX 15, France.

The liver DNA of a West African patient with advanced hepatocellular carcinoma has been cloned. The liver was HBsAg negative by immunofluorescence, whereas the serum was positive for the same antigen. The cellular DNA contained a minimum of 5 HBV genomic fragments as evidenced by Southern blotting. The DNA was partially digested by MboI, fractionated on a sucrose gradient and the 10-20 kilobase fraction pooled. Subsequently, ligation was made into the BamHI vector λ471. We have isolated several genomic clones containing integrated HBV sequences. The genomic organization of the clones has been deduced. The clone λIA22 has been analysed in most detail and most of the subsequent data refer to it. The 5' site of integration lies very close to the site of the nick in the free viral DNA. Thus the organization of the two viral antigen genes is : integration 5'-core gene-surface gene-core gene-3' integration site. Clone λIA22 contains approximately 12 genomes at HBV DNA. There are no detectable inversions, deletions, or many other arrangements of HBV sequences as determined by electron microscopy. The generation of multiply integrated HBV genomes will be discussed. Restriction enzyme mapping of the HBV sequence shows that it is unique and is not described by any of the four known maps. However there are more sites in common with the ad than the ay subtype - suggesting than it is an ad polymorph. We have cotransformed the phage DNA and the cloned HSV-1 thymidine kinase gene into mouse Ltk^- cells by the calcium phosphate technique. After selection on HAT medium, we obtained several cloned one of which expressed HBsAg in the supernatant. After general cycles of purification the HBsAg activity was shown to band of 1,22 gml^1 in CsCl - exactly as do human serum HBsAg particles. Immunoelection microscopy confirmed the existence of HBsAg particles which could be aggregated by prior adsorption with purified rabbit anti-HBsAg serum.

0637 STATE OF THE HEPATITIS B VIRUS (HBV) DNA IN THE LIVER AND THE SERUM OF PATIENTS WITH HBV RELATED LIVER DISEASE. Christian Bréchot, Michelle Hadchouel, Jacques Scotto, Françoise Degos, Anne Dejean and Pierre Tiollais, Institut Pasteur, 75015 Paris, France.

We used the Southern transfer hybridization technique to study the presence and the state of HBV DNA in the liver and serum of patients with hepatocellular carcinoma (HCC) (35 patients), chronic hepatitis (40 patients) and acute fulminant hepatitis (5 patients). Two different states were distinguished : the first is characterized by the presence of free viral DNA in the liver with viral DNA and HBeAg in the serum ; viral DNA sequences integrated in the cellular DNA are also present in the liver at least in some patients The second state is characterized by the detection of only integrated HBV DNA without viral DNA and HBeAg in the serum. The liver DNA restriction patterns observed in patients without tumour suggests the existence of specific integration sites for the viral DNA in the cellular DNA. Integrated HBV DNA sequences were also found in 5 patients with acute fulminant hepatitis and in early HBV chronic carriers suggesting that integration of HBV DNA is an early event in the course of the disease. In addition the viral DNA was also detected in the liver of patients with chronic hepatitis although no HBV serological marker was detectable, and in the liver of patients with HCC and alcoholics cirrhosis or haemochromatosis.

0638 BONE MARROW ORIGIN OF THYMIC LYMPHOMA IN AKR MICE, Esther F. Hays and Haruko Takeuchi, University of California, Los Angeles, CA 90024

Bone marrow cells from 1-month-old AKR mice give rise to thymic lymphomas when grafted to AKR x DBA/2 F_1 hybrid mice. This was determined by inoculating $2x10^7$ AKR bone marrow cells IV into 400 rad-treated 6-8 wk old recipient animals. Six of 12 mice given AKR bone marrow developed thymic lymphoma between 158 and 244 days after inoculation. These lymphomas were of AKR cellular origin. Nine control mice were treated with 400 rads but not given cells. Lymphoma developed in 3 animals. These lymphomas were of hybrid cellular origin.

The experiment was repeated using bone marrow from 3-week-old mice treated when newborn with a cloned AKR-derived lymphomagenic virus, SL3-3. 15 of 16 marrow-inoculated mice developed thymic lymphoma 87-150 (median 91) days later. Twelve of these tumors were of AKR type, one was of hybrid type and tests on the other two are not yet complete. Thus, it is shown that marrow progenitors from AKR mice treated with a lymphomagenic virus can also give rise to lymphomas. This system is also being used to study lymphomagenic potential of progenitor cells of virus-treated AKR mice after various conditions of long-term culture.

These studies demonstrate that the AKR thymic environment is not necessary for lymphomagenesis and that cells derived from AKR bone marrow of normal and virus-treated AKR donors will develop lymphoma in the hybrid recipients in preference to the hybrid progenitors. These lymphomas develop after latent periods similar to those found in the host animals.

0639 THE EARLY STAGE OF FV ERYTHROLEUKEMIA:A TENTATIVE PATHOGENETIC MODEL. C. Peschle^°, G. B. Rossi^,A. Covelli°,G. Migliaccio°,G. Mastroberardino+. ^Ist. Superiore Sanità,°Univ. of Rome,Rome;+Univ. of Naples,Naples,Italy. Abbreviations:Friend Virus,polycythemic or anemic,FVP or FVA;Friend murine leukemia Virus,F-MuLV;Spleen focus-forming Virus,polycythemic or anemic,$SFFV_P$ or $SFFV_A$;Spleen colony-forming unit(s),CFU-S;Burst-,colony-forming unit(s),erythroid,BFU-E,CFU-E;Burst-promoting activity,BPA;Erythropoietin,Ep.

FVP or FVA injection in adult DBA/2 mice causes a rise of BPA,documented in vitro as early as on day 5-7,via mechanisms of both "intrinsic" (hypersensitivity of BFU-E to BPA) and "extrinsic" (enhanced BPA release,particularly by splenocytes) type.The rise of BFU-E cycling in FV-infected mice is presumably induced by the BPA elevation. FVP (but not FVA) injection also causes a rise of Ep or Ep-like activity,via intrinsic (marked hypersensitivity of CFU-E to Ep, up to apparent independence of it) and possibly extrinsic (enhanced Ep release) mechanisms.

It is suggested that the F-MuLV component in FVP and FVA is largely responsible for the BPA rise,while $SFFV_P$ selectively causes the elevation of Ep activity.The pathogenetic sequence in the early stage of FV leukemias might include:(1) increased BPA release,possibly via T-lymphocyte-macrophage infection.(2) Enhanced BFU-E (and possibly CFU-S) cycling.(3) FV infection of cycling BFU-E (and CFU-S).(4) Transformation of BFU-E (and CFU-S), expressed by their hypersensitivity to BPA.(5) Sustained elevation of BFU-E (and CFU-S) cycling and erythroid differentiation,leading to massive erythropoietic expansion.In FVP-infected mice,$SFFV_P$ may transform CFU-E, as indicated by their hypersensitivity to Ep:this would render effective the enhanced erythropoietic differentiation, thus leading to polycythemia.

Poster Session III

0640 STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN TWO HIGHLY RELATED TRANSFORMATION SPECIFIC PROTEINS IN PRC II TRANSFORMED CELLS. Becky Adkins and Tony Hunter, TVL, Salk Institute, P.O. Box 85800, San Diego, Calif. 92138.

Cells transformed by the avian sarcoma virus, PRC II, were found to contain two transformation specific phosphoproteins of 110,000 d (P110) and 105,000 d (P105), present in approximately equimolar amounts. Pulse chase analysis revealed that P110 was derived from P105 within 15 min of its synthesis by a post-translational modification. P110 and P105 were found to differ by several criteria. First, when PRC II transformed cells were treated with a buffered solution containing a non-ionic detergent, P105 was found almost exclusively in the detergent soluble fraction whereas P110 remained with the detergent insoluble "cytoskeletal" fraction. Secondly, analysis of ^{32}P-labelled P110 and P105 prepared in this manner showed that P110 contained a greater ratio of phosphotyrosine to phosphoserine than P105 and that P110 had two phosphotyrosine containing tryptic peptides not found in P105. Finally, when protein kinase assays were performed in immunoprecipitates of detergent extracted PRC II transformed cells, only that fraction which contained P110 displayed a tyrosine specific protein kinase activity. These results suggest that phosphorylation at specific tyrosine residues or sub-cellular location or both are important in the regulation in vivo of the PRC II associated tyrosine specific protein kinase activity.

0641 ABELSON VIRUS TARGETS IN THYMUS Wendy D. Cook, Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia

Although Abelson murine leukemia virus was derived from the T leukemogenic Moloney virus, its transformants in vivo and in vitro are generally of B or null lymphoid phenotype, and occasionally myeloid. In order to test whether Abelson can rapidly transform T cells, Abelson virus has been injected intrathymically into mice. Thymomas appear in 25-40 days. Such tumors induced in B/C mice consist largely or exclusively of Thy 1 negative cells, whereas Thy 1 positive tumors arise in C57Bl/Ka mice. Tissue culture lines have been derived and cloned. Abelson gene products and biological activity have been demonstrated. Cell surface markers are being characterized.

This work demonstrates that Abelson virus has targets in the thymus, which suggests that its apparent change in tissue tropism is not absolute.

0642 **SITE DIRECTED MUTAGENESIS WITHIN THE src GENE OF ROUS SARCOMA VIRUS, Debra Bryant and J.T. Parsons, University of Virginia, Charlottesville, VA 22908.**

Mutations have been introduced into the src gene of molecularly cloned Rous sarcoma viral DNA at two restriction endonuclease sites, BglI and BglII. The BglI site spans the sequence for ... ala_{430} pro glu ala_{433} ... . Two point mutants have been identified which have sequence changes ala_{430} val_{430} and ala_{433} thr_{433}. Transfection of chicken CE cell with both mutants resulted in the synthesis of mutant $pp60^{src}$ with no concommitant change in cellular morphology. The proximity of these mutations to the phosphotyrosine of $pp60^{src}$ (tyr_{416}) suggests that this region is important for functional $pp60^{src}$. Deletion mutants have been constructed at the BglII site which encompasses the coding sequence for ... lys_{203} ilu tyr ... . The mutant, CH 119, contains a deletion of 185 base pairs and codes for a protein with a molecular weight of approximately 53,000. Chicken cells infected with CH 119 are temperature sensitive for transformation. A detailed analysis of the different parameters of transformation will be presented.

0643 ACTIVITY OF THE TRANSFORMING PROTEIN OF FUJINAMI SARCOMA VIRUS. Tony Pawson, Geraldine Weinmaster, and G. Steven Martin*. Dept. of Microbiology, Univ. of British Columbia, Vancouver, B.C. V6T 1W5, and *Dept. of Zoology, University of California, Berkeley, CA 94720. Fujinami sarcoma virus (FSV) is a replication-defective transforming avian retrovirus which encodes a single gene product of 130-150K (p140). This protein has at its amino-terminus a sequence synthesized from a defective gag gene while its carboxy-terminus is encoded by the fps sequence. The p140 is phosphorylated at tyrosine residues and is associated with a tyrosine specific protein kinase activity in vitro. In variants of FSV which are ts for transformation the phosphorylation of p140 and of cellular proteins at tyrosine and the p140-associated protein kinase activity are co-ordinately ts with the transformed phenotype. Immunoprecipitates of p140 also contain the 50K and 89K cellular proteins which are specifically associated with RSV $pp60^{src}$. The protease activity of virion p15 cleaves p140 into an N-terminal 33K gag-encoded fragment and a 120K C-terminal fps-encoded fragment. All the tyrosine phosphorylation sites are located within the fps-encoded region of p140. Tryptic phosphopeptide analysis of p140 shows that one major site of tyrosine phosphorylation is conserved between several variants of FSV; and that this peptide is cleaved further by V8 protease under conditions where the protease is specific for glutamic acid residues. The sequence around the phosphorylated tyrosine will be presented. Considerable variation in the other sites of tyrosine phosphorylation has been observed between different strains and mutants of FSV, and these variations do not appear to affect the transforming activity of the p140 . FSV p140 partially purified by ion-exchange chromatography retains a tyrosine-specific kinase activity. These data indicate that these protein kinase reactions are probably responsible for FSV-induced transformation.

0644 EVIDENCE THAT THE DIVERSE TUMOUR SPECIFIC TRANSPLANTATION ANTIGENS OF MICE ARE MuLV ENVELOPE GENE PRODUCTS. Gerard I. Evan, Edwin S. Lennox; MRC Laboratory of Molecular Biology, Hills Rd., Cambridge CB2 2QH, England.

To study the molecular basis for the diverse Tumour Specific Transplantation Antigens (TSTA) of chemically induced mouse tumours, we made monoclonal antibodies (McAbs) against a chemically induced sarcoma of a B10 mouse, MC6A. One of these McAbs (A1/4B1) showed a high specificity for MC6A amongst a wide variety of tumour lines and normal cells of similar and differing genetic backgrounds. On this basis, we consider the A1/4B1 antigen a good candidate for the MC6A TSTA.

By depletion of MC6A cell lysates with sheep anti Friend MuLV gp70 serum, and by peptide mapping, we have shown that the A1/4B1 antigenic determinant is expressed on an MuLV gp70-like molecule. Moreover, this determinant is not found on any normal tissue of the B10 strain of mouse. To test the association of the A1/4B1 antigen with the MC6A TSTA, we have selected several tumour lines purely on the basis of whether or not they express the A1/4B1 antigen, and have tested them in a transplantation rejection assay against the tumour MC6A. With 5 tumours tested, there is complete correlation between the presence of the A1/4B1 antigen and the ability to immunise against MC6A. Thus all evidence so far supports the possibility that the TSTA of the tumour MC6A is a rare polymorphic determinant on a MuLV gp70. We suggest that in addition to the known role of MuLV recombination in the generation of acute transforming viruses, there is the possibilty that recombinant MuLV gene products might provide the basis for TSTA diversity.

0645 INTRACELLULAR TARGETING OF HERPES SIMPLEX VIRUS GLYCOPROTEINS, David C. Johnson and Patricia G. Spear, University of Chicago, IL 60637

Herpes simplex virus type 1 (HSV-1) specifies the synthesis of 4 antigenically distinct glycoproteins designated gB, gC, gD, and gE which are constituents of plasma membranes and also participate in the envelopment of viral nucleocapsids on nuclear membranes in infected cells. To investigate the mechanisms by which viral glycoproteins are targeted to specific cellular organelles we examined the intracellular transport, post-translational processing and assembly of HSV-1 glycoproteins. Cellular membranes of infected HEp-2 cells labelled with ^{35}S-methionine, ^{14}C-glucosamine, or ^{3}H-palmitate were fractionated on sucrose gradients, the viral glycoproteins solubilized with detergents and precipitated with monoclonal antibodies. Processing of N-linked oligosaccharides was monitored by use of endoglycosidase H and the attachment of O-linked oligosaccharides was followed by alkaline elimination and by the use of N-acetyl galactosaminyl oligosaccharidase. Proteases were applied both to cell surfaces and to microsomal preparations in attempts to follow the transport of viral glycoproteins. The results of these experiments suggest that the HSV-1 glycoproteins move from the rough endoplasmic reticulum (RER) to nuclear membranes where envelopement begins. Fully or partially enveloped virions move from the periplasmic space of the nucleus to the RER then on to the Golgi apparatus where N-linked oligosaccharides are processed and fatty acid molecules are attached to gB. Virions are then carried to the cell surface where a large proportion of the particles remain attached possibly acting to fuse cells. The pathway of egress of HSV-1 virions was confirmed in experiments with the ionophore monensin.

0646 MONOCLONAL ANTIBODIES DISTINGUISH FORMS OF P21 ENCODED BY DIVERGENT MEMBERS OF THE RAS ONCOGENE FAMILY, Mark E. Furth, Lenora J. Davis, Barbara Fleurdelys and Edward M. Scolnick, Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, MD 20814

The ras transforming genes carried by the Harvey and Kirsten murine sarcoma viruses (HaSV and KiSV) derive from two distinct members of a family of conserved cellular genes. We have used monoclonal antibodies (McAbs) to study relationships among the 21,000 dalton protein products (p21) encoded by viral and cellular ras genes. Eight McAbs to determinants on the p21 of HaSV were identified by specific immunofluorescence on transformed cells, immunoprecipitation of guanine nucleotides bound to partially purified p21, and immunoprecipitation of metabolically labeled p21. Three of these McAbs cross-react strongly with the p21 encoded by KiSV. The same three McAbs also cross-react strongly with the predominant cell-encoded p21 species expressed in a variety of rodent cell lines, including a line of primitive hematopoietic cells (416B) that produce exceptionally high levels of the protein. Thus, the majority of endogenous p21 in rodent cells appears to be encoded by a ras gene more closely related to the transforming gene of KiSV than to that of HaSV. This conclusion was confirmed directly for 416B cells by hybridization of Ha and Ki-specific ras DNA probes to mRNA blots (R. Ellis, D. DeFeo, M. Furth, P. Tsichlis, et al.). One McAb to a determinant shared by all known forms of p21 can bind the protein even after denaturation, and may recognize an amino acid sequence critical for guanine nucleotide binding.

0647 STUDIES OF BSB, A MURINE ERYTHROLEUKEMIA VIRUS COMPLEX DISTINCT FROM FRIEND VIRUS, Natalie M. Teich and Janice Rowe, Imperial Cancer Research Fund, London WC2A 3PX, England

The polycythemia and anemia inducing strains of Friend erythroleukemia virus complex do not produce erythroleukemia in adult C57BL mice, primarily due to the effect of the Fv-2^{rr} gene. However, by blind passage of the polycythemia strain in neonatal C57BL mice, a new variant, BSB, capable of inducing erythroleukemia and spleen foci in adult C57BL mice was developed (Steeves et al., Int. J. Cancer 5:346, 1970). Our initial investigations of the BSB stock by SDS-polyacrylamide gel electrophoresis indicated a very complex pattern of intracellular virus-specific proteins, compatible with the idea of multiple virus components. Using end-point dilution and single cell cloning, we derived cell clones containing different viruses. The protein patterns of the cell clones showed marked diversity following immunoprecipitation with virus-specific antisera: both replication-competent and replication-defective viruses fell into several distinct classes. Neither the uncloned BSB nor the cloned derivatives synthesize gp55, the env gene recombinant protein considered the hallmark of the erythroleukemia-inducing replication-defective spleen focus forming virus component of Friend virus complex. Analysis of the BSB viruses for tumorigenicity in neonatal and adult mice of several strains revealed that the erythroleukemia-inducing component was associated with the synthesis of two intracellular proteins, a glycoprotein of 43,000 daltons and a nonglycosylated protein of 50,000 daltons, both of which contain viral env antigens. The tumorigenicity assays also indicated the presence of host gene(s), other than Fv-2^{rr}, that mediate susceptibility to BSB-induced erythroleukemia.

0648 CONSERVED AND OPTIONAL SEQUENCES IN THE TRANSFORMING GENES OF AVIAN RETROVIRUSES, Peter Duesberg, Klaus Bister, Wen-Hwa Lee, Michael Nunn, and Terry Robins, Department of Molecular Biology, University of California, Berkeley, CA 94720.

Eight groups of transforming onc genes can be distinguished among avian sarcoma and acute leukemia viruses based on specific RNA sequences, which are unrelated to essential virion genes. The products of some of these, e.g., the src gene of Rous virus and the onc gene of myeloblastosis virus are entirely encoded by specific RNA sequences (type 1), while the products of all others are hybrids (type 2) encoded by specific and virion gene-related sequences like Δgag or Δgag and Δpol. By comparing nucleotide sequences, the type 1 onc genes of 10 different Rous strains were found to be 98% conserved, consistent with the presumed kinase function of the src gene product. By contrast, type 2 onc genes of the myelocytomatosis virus MC29 subgroup and of the Fujinami sarcoma virus subgroup were found to contain conserved and optional sequence elements. We speculate that the existence of conserved and optional sequence elements signals multiple functional domains. We are now mapping conserved and optional elements in type 2 onc genes and investigating whether the cellular prototypes of their specific sequences also contain these elements.

0649 EXPRESSION OF THE MO-MSV TRANSFORMING GENE IN TRANSFORMED CELLS, Jackie Papkoff, Tony Hunter and Inder Verma, Salk Institute, P.O. Box 85800, San Diego Ca. 92138

Moloney murine sarcoma virus (Mo-MSV) induces fibrosarcomas in animals and transforms fibroblasts in vitro. We previously demonstrated that the transforming gene, v-mos^{Mo}, within Mo-MSV virion RNA could be translated in vitro to yield an overlapping set of four proteins of approximately 37K, 33K, 24K and 18K. Analysis of these in vitro products showed that each one was initiated independently from AUG codons within the v-mos^{Mo} gene and was translated in a reading frame predicted from the v-mos^{Mo} DNA sequence. An antiserum against a synthetic peptide corresponding to the C-terminus of the predicted v-mos^{Mo} gene product precipitates all four in vitro v-mos^{Mo} proteins. We have used this antiserum (anti-C3) to identify and characterize the mos proteins from MSV transformed cell lines. Anti-C3 recognizes a doublet of approximately 37K from ^{35}S-Methionine labeled Mo-MSV transformed producer cells which, by peptide mapping, is almost identical to the 37K in vitro mos gene product. Immunoprecipitation of these same cells labeled with ^{32}P reveals a single phosphoprotein which appears to comigrate with the upper band in the ^{35}S doublet. Pulse-chase experiments show that the lower band in the ^{35}S doublet is the primary translation product which is modified, probably by phosphorylation, to yield the upper band. This protein has at least six phosphorylated sites, all of which contain phosphoserine. A similar mos-specific protein can be immunoprecipitated from Mo-MSV cDNA transformed nonproducer cells and HT1-MSV transformed cells but not from normal NIH 3T3 cells or Mo-MLV infected cells. These mos proteins are present at very low levels in transformed cells. Cells newly infected with Mo-MSV, however, transiently contain much higher levels of mos protein.

0650 SELECTIVE TROPISMS OF MCF VIRUSES FOR LYMPHOCYTE SUBPOPULATIONS, Miles W. Cloyd, NIH, NIAID, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, Hamilton, MT 59840. An initial and probably critical event involved in genesis of leukemia by murine leukemia viruses (MuLV) is the selective infection of certain cells with particular viruses. This event was studied by first determining what cells were targets for lymphomagenic and nonlymphomagenic MCF viruses. Two lymphomagenic viruses (AKR-247, C58L1) were found to infect and replicate selectively in cells only present in thymus of AKR mice. These cells expressed Thy-1, did not adhere to plastic or nylon wool, and were sensitive to lysis by glucocorticoids *in vivo*. This suggested that they were probably the immature lymphocytes present in the thymic cortex. There was no characteristic pattern of expression of either Lyt-1 or Lyt-2 antigens on these cells, which was similarly reflected by the lymphoma cells induced by these viruses. Nonlymphomagenic MCF viruses isolated from NFS.*C58V-1* or NFS.*C58V-2* mice infected lymph node, spleen, and bone marrow but not thymus or some non-lymphoid organs tested. The target cells for these viruses were Thy-1 negative and plastic nonadherent, but they did adhere to nylon wool and were stimulated *in vitro* by LPS but not Con A. This cell was probably a B-lymphocyte. These findings demonstrate that, in contrast to ecotropic viruses which can infect many types of cells in the mouse, specific cellular tropism often exist for MCF viruses. Furthermore, since it seems likely that these tropisms are determined by specific cellular receptors complimentary to the respective MCF virus, the existence of various differentiation-associated MuLV cellular receptors is suggested.

0651 TRANSFORMATION SPECIFIC PROTEIN IN ADHESION SITES OF RSV-INFECTED CHICKEN EMBRYO FIBROBLASTS, John Blenis and Susan P. Hawkes, Michigan Molecular Institute, Midland, MI 48640, and Michigan State University, East Lansing, MI 48824.

We have detected a novel protein whose synthesis is elevated in the adhesion sites of chicken embryo fibroblasts infected with a temperature-sensitive mutant of Rous sarcoma virus. Cells are removed from culture dishes with EGTA and proteins remaining in the substratum-attached adhesion sites analyzed by gradient polyacrylamide gel electrophoresis. Transfer of cells to the permissive temperature results in a dramatic increase in a protein of approximate molecular weight, 21,000, during the early stages of transformation. Synthesis of the 21K protein peaks at 8 hours following a temperature shift and declines to 1.3-fold that of normal cells by 16 hours. Turnover of the 21K protein is slow. It is not the product of normal cell protein degradation or processing upon shift to the permissive temperature. Characterization reveals metabolic labeling by amino acids, but not mannose, and lack of phosphorylation. Furthermore, it is resistant to extraction with Triton X-100, Nonidet P40, deoxycholate, octyl glucoside, EGTA and urea, but can be removed from the substratum by NP40 containing high salt or 1% mercaptoethanol, and sodium dodecyl sulfate. In addition, we have found that removal of cells from the substratum with trypsin leaves the 21K protein on the substratum whereas the proteases present in pronase do not. The functional significance of this transformation specific protein is currently under investigation.

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0652 ANALYSIS OF *MYC* GENE PRODUCTS IN ALV-TRANSFORMED LYMPHOID CELLS AND IN MH2 TRANSFORMED CELLS, C. Pachl, M. Linial, R. Eisenman, and B. Biegalke, Hutchinson Cancer Research Center, Seattle, Washington 98104

The defective avian leukosis virus MC29 encodes an oncogene, *myc*, which is expressed in transformed cells as a *gag-myc* polyprotein, P110. *Myc* sequences are also present in normal cells, however a cellular *myc* protein has not been identified. Using a DNA clone of the cellular *myc* gene, c-*myc*, hybrid-selected translation experiments have been carried out with RNAs from normal and transformed cells in order to detect a *myc* protein. When RNA from RP9 cells, a line of ALV transformed lymphoid cells which have elevated levels of c-*myc* mRNA (Hayward, W.S., et al., Nature 290:475-480, 1981), is hybrid selected with c-*myc* DNA a 57K protein is specifically detected.

The expression of *myc* proteins in quail cells transformed by MH2, a member of the MC29 group of viruses, is also being examined. A variant MH2 virus has been isolated which does not express the MH2 gag-*myc* polyprotein (P100). When RNA from cells transformed by this virus, MH2VS3, is hybrid selected by c-*myc* DNA, a 57K protein is also detected. This result suggests that unlike MC29, MH2 may also express a non-*gag* containing *myc* protein similar to the 57K cellular *myc* protein.

0653 A VARIANT MOLONEY MURINE SARCOMA VIRUS CODING FOR A 85,000 DALTON gag-mos POLYPROTEIN, R. B. Arlinghaus, E. C. Murphy, Jr., R. Junghans and W. S. Kloetzer, Dept. Tumor Virology, Univ. Tex. M.D. Anderson Hosp. & Tumor Inst., Houston, TX, 77030.
The 6m2 clone of NRK cells infected with a temperature-sensitive (ts) transformation mutant (ts110) of Moloney murine sarcoma virus (MuSV) contains two mouse src (mos)-containing RNAs of about 4.0 and 3.5kb. These RNAs are also present in virions. When they are hybridized to cloned MuSV-124 DNA, each contain a large deletion. The 4.0kb RNA results from a deletion of 1.5kb that begins in p30 and ends near the start of the v-mos gene. The 3.5kb RNA has suffered a deletion 400 bases upstream from that found in the 4.0kb RNA. Translation experiments indicate that the 3.5kb RNA codes for $P85^{gag\text{-}mos}$, whereas the 4.0kb RNA yields $P58^{gag}$. Both the 3.5 and 4.0kb RNAs may be primary transcripts. However, it remains to be determined whether the 3.5kb RNA arises by a splice mechanism. Subgenomic poly(A)-containing RNAs yield mos-related P33 and P23 (Lyons et al., Virology 105: 60, 1980). $P85^{gag\text{-}mos}$ has tyrosine and cysteine tryptic peptide patterns consistent with the known mos sequence. These results indicate that the 3.5kb MuSV RNA codes for $P85^{gag\text{-}mos}$ as a result of juxtaposition of open gag and mos reading frames. Intracellular forms of $P85^{gag\text{-}mos}$ and $P58^{gag}$ contain NaOH-resistant phosphate, only phosphorylated $P58^{gag}$ being detected at the restrictive temperature. Interestingly, an acidic protein kinase, not found in MuSV-349 and different in properties from the MuLV kinase, has been isolated from ts110 virions. Such virions also appear to contain phosphorylated $P85^{gag\text{-}mos}$. The relationship of the ts110 kinase to $P85^{gag\text{-}mos}$ is being investigated.

0654 ANALYSIS OF VIRUS-SPECIFIC RNAs AND POLYPEPTIDES IN REVERTANT AND RESCUED ts CELL CLONES OF Mo-MuSV ts110, E. C. Murphy, J. P. Horn, D. G. Blair, and R. B. Arlinghaus, Dept. Tumor Virology, Univ. Texas M. D. Anderson Hosp. & Tumor Inst., Houston, Texas 77030.
Virus-specific RNAs and polypeptides in nonproducer cell lines infected with ts110 virions rescued with SSAV were studied. Lines 204-2f6 and 204-2f8 retained the ts phenotype at both 33° and 39°. Line 204-3 had reverted to the transformed phenotype at both temperatures, and was studied along with a spontaneous revertant line, 54-5A4. The parental cell line, 6m2, transformed at 33° and normal at 39°, contained two virus-specific RNAs at 33°, 4kb and 3.5kb in size. However, only the 4kb RNA (in greatly reduced quantity) was found at 39°C, correlating well with the observation of virus-specific polypeptides $P58^{gag}$ and $P85^{gag\text{-}mos}$ in 6m2 cells at 33° with only the $P58^{gag}$ was found at 39°. Cell clones 204-2f6 and 204-2f8, retaining the ts phenotype, contained the 4kb (P58 coding) RNA at both 33° and 39°, but only 204-2f6 appeared to contain the 3.5kb RNA (P85 coding) at 33°. However, neither cell line contained a $P85^{gag\text{-}mos}$. The revertant cell lines, 54-5A4 and 204-3 contained both the 4.0kb and 3.5kb RNA. In these cells, interestingly, a $P100^{gag\text{-}mos}$ polypeptide which appeared to represent the sum of $P58^{gag}$ and $P38^{mos}$ peptide sequences was found instead of $P85^{gag\text{-}mos}$. Thus, the $P85^{gag\text{-}mos}$ polyprotein coded for by a 3.5kb RNA whose truncated gag sequences are fused to the mos sequences as a single open reading frame appears to produce a ts phenotype. In the transformed revertants, however, either a back mutation to produce a normal mos gene or inclusion of the C-terminal of p30 into the gag-mos fusion protein, yielding a $P100^{gag\text{-}mos}$, appears to produce a revertant phenotype.

0655 THE LONG TERMINAL REPEAT OF MOUSE MAMMARY TUMOR VIRUS CONFERS HORMONE INDUCIBILITY TO THE EXPRESSION OF THE PROVIRAL AND CHIMERIC THYMIDINE KINASE GENES, Bernd Groner, Helmut Ponta, Ursula Rahmsdorf and Nancy Hynes, Institute of Genetics, Nuclear Research Center, Karlsruhe, Federal Republic of Germany
An endogenous proviral gene of mouse mammary tumor virus (MMTV) has been isolated by molecular cloning. This provirus is not active in virus production in the animal. Following transfection into cultured cells the proviral gene is transcribed in a glucocorticord responsive fashion and virus particles are produced. To define the nucleotide sequence responsible for glucocorticord induction a fragment of the proviral DNA containing the MMTV promoter (LTR) was recombined in vitro with the thymidine kinase (tk) gene of herpes Simplex Virus in a syn orientation. In cells transfected with the chimeric gene a fusion mRNA of LTR and tk sequences is expressed in a hormone inducible way. Deletions in the LTR sequence showed that the entire LTR (1328 nucleotides) is not necessary to confer hormone inducibility. The analysis of chimeric LTR - tk genes in which the distance between the LTR and the tk sequences was increased and the orientation with respect to their polarity of transcription was inverted revealed an enhancing effect of the LTR on tk transcription. The enhanced tk transcripts are not fusion mRNAs and are not hormone inducible. Both effects of the LTR, hormone inducible transcription of directly adjacent DNA and enhancement of transcription from promoters in their wider vicinity could be involved in mammary tumor induction through proviral insertion.

0656 SUBCELLULAR LOCALIZATION OF VIRAL TRANSFORMING PROTEINS AND THEIR CELLULAR TARGETS, Paul Moss, Tom Gilmore, Kathryn Radke, and G. Steven Martin, University of California Berkeley, California 94720

The transforming protein of RSV, pp60^{src}, is known to be membrane associated. We have used cell fractionation techniques to determine the subcellular localization of the transforming protein, p140, of Fujinami Sarcoma Virus (FSV). ^{35}S-methionine labelled chick cells transformed with a wild-type, temperature-resistant strain of FSV were homogenized in a hypotonic medium and fractionated into a nuclear pellet, a 100,000 x g pellet (P100), and a soluble fraction (S100). Analysis of these fractions by immunoprecipitation demonstrated that more than 80% of the p140 was in the P100 fraction. Further fractionation of the P100 material on a discontinous sucrose gradient revealed that p140 partitioned with the plasma membrane In contrast, in cells transformed with a temperature-sensitive strain of FSV, 25-40% of the p140 was in the soluble fraction at the permissive temperature and 50-60% was soluble when these cells were shifted to the nonpermissive temperature. p140 also complexes with the cellular phosphoproteins, pp90 and pp50; our fractionation experiments indicate that the complex, in contrast to the monomeric p140, is soluble. Furthermore, the 36 kilodalton phosphoprotein which has been identified as a cellular target for phosphorylation by viral transforming proteins was localized to the P100 fraction. These and other results suggest that membrane association of viral transforming proteins may play a role in viral transformation.

0657 PROTEIN PHOSPHORYLATIONS ASSOCIATED WITH ONCOGENIC TRANSFORMATION BY AVIAN SARCOMA VIRUS UR2, E. Maytin, D.A.Young, M. Notter and P.C. Balduzzi, Depts. of Medicine and Microbiology, Univ. of Rochester Sch. of Med. & Dent., Rochester, New York 14642.

UR2 is a newly described replication-defective avian sarcoma virus (Balduzzi, et al., J. Virol.40:268, 1981) with a transforming insert ros unrelated to any other known avian retrovirus oncogenes (Shibuya M. et al., J. Virol. In Press). UR2 codes for a single species of RNA (24S) which possesses unique oligonucleotides flanked by oligonucleotides common to its helper virus UR2AV (Wang, L.-H. et al., J. Virol. In Press). This RNA codes for p68 ros, a protein kinase precipitable from cell lysates with antiviral or anti-gag sera. p68 phosphorylates itself on tyrosine *in vitro* and also phosphorylates tyrosine residues on added substrates (Feldman R., et al., J. Virol, In Press).

In vivo UR2 phosphorylates the "36K" protein first detected by Radke and Martin (P.N.A.S. USA 76:5212, 1979) as shown for other ASV (Cooper and Hunter, Mol. Cell Biol. 1, 394, 1981). To search for further changes in phosphorylation of cellular proteins by UR2, ^{32}P-labelled cell lysates from UR2-, UR2AV-, RSV-infected, or uninfected chick embryo fibroblasts were separated by "giant gel" 2-D electrophoresis, which affords a ≈ 3-fold increase in resolution compared to standard 2-D gels (Voris and Young, Anal. Biochem. 104, 478, 1980). Changes specific for transformation (UR2 vs. UR2AV) as well as virus invection-specific changes (UR2AV vs. control) were found in a wide range of pH regions. Some changes occurred in both UR2 and RSV transformed cells, while others appear to be specific for transformation by UR2 or RSV. (Supported by NIH grants: AM16177, CA15716, and GM07356.)

0658 SUBCELLULAR LOCALIZATION OF PUTATIVE TRANSFORMING PROTEINS OF AVIAN ACUTE LEUKEMIA VIRUSES, Holly D. Abrams, Larry R. Rohrschneider, and Robert N. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Avian cells transformed by the acute or defective leukemia viruses (DLVs), avian myelocytomatosis virus (MC29) or avian erythroblastosis virus (AEV), synthesize polyproteins consisting of sequences derived from both the gag and the unique onc regions. These polyproteins are believed to be involved in transformation. Two approaches have been taken to determine the location of the gag-related proteins in avian cells transformed by DLVs: subcellular fractionation and indirect immunofluorescence. Both lines of evidence indicate that in a variety of MC29-transformed quail target cells, the P110$^{gag-myc}$ protein is associated with nuclei. In contrast, the majority of AEV P75 $^{gag-erb}$ is found in the cytoplasm and in the combined cytoplasmic and membrane fraction of nonproducer AEV-transformed chick embryo fibroblasts in immunofluorescence and subcellular fractionation studies, respectively. Therefore, the gag-related v-onc proteins of MC29 and AEV exhibit an intracellular distribution distinct from each other. MH2 virus is considered a member of the MC29 class, although some differences exist in the myc region of the genomes, in the predominant type of neoplasms induced, and in target cell preferences in vitro. In MH2-transformed quail bone marrow cells, the P100 $^{gag-myc}$ protein was localized predominantly in the combined cytoplasmic and membrane fraction. The particular sites of localization of transforming proteins may be relevant to the oncogenic spectrum and mechanisms of transformation of avian acute leukemia viruses and avian sarcoma viruses.

0659 ERYTHROBLASTOSIS VIRUS E26 AND MYELOBLASTOSIS VIRUS AMV SHARE TRANSFORMATION-SPECIFIC RNA SEQUENCES, BUT DIFFER IN GENETIC STRUCTURE AND GENE PRODUCTS. Klaus Bister*, Michael Nunn+, Peter Duesberg+, and Carlo Moscovici#. *Max-Planck-Institut für Molekulare Genetik, D-1000 Berlin 33, Germany; +Department of Molecular Biology, University of California, Berkeley, California 94720; #Virus Laboratory, VA Hospital, Gainesville, Florida 32601.

Avian acute leukemia virus E26 causes erythroblastosis in chicken and quail, while AMV induces a myeloid leukemia. Hence, the two viruses transform target cells of different haematopoietic lineages.Nevertheless, AMV and E26 RNAs share transformation-specific sequences. However, the genetic designs and gene products of the two viral RNAs are different. E26 RNA measures 5.7 kilobases(kb) as compared to the 7.5kb RNA of AMV, and hybridizes to AMV-specific proviral DNA. Hybridization of size-selected poly(A)-containing E26 RNA with AMV-specific DNA suggests that these sequences are located in the 5'-half of the E26 genome as opposed to a 3'-location in AMV RNA. In nonproducer cells transformed in vitro by E26 a gag-related, nonstructural protein of 135,000 dalton(p135) is found. No Pr76gag or Pr180gag-pol proteins, which are present in AMV nonproducer cells, are observed. p135 is also found in immunoprecipitates from the immature blood cells obtained from chicken with E26-induced erythroblastosis, and its relative amount correlates with the ratio of E26 to helper RNA in virions releasedbythese cells. p135 is phosphorylated, but not glycosylated, and antigenically related to the gag, but not the pol or env genes. It seems to be coded for by a partial gag gene and E26-specific RNA sequences presumably including those shared with AMV. Hence, AMV and E26 appear to use different strategies for the expression of related onc sequences: AMV presumably encodes a transforming protein via a subgenomic mRNA, while E26 codes for a gag-related polyprotein.

0660 ACQUISITION AND EXPRESSION OF CELLULAR SEQUENCES BY RETROVIRUSES,Ronald Swanstrom, Richard C. Parker, Harold E. Varmus and J. Michael Bishop, University of California, San Francisco, California 94143.

We have been studying the mechanism by which retroviruses capture and express cellular sequences, specifically the v-src gene of Rous sarcoma virus. Expression of the v-src gene product, $pp60^{v\text{-}src}$, leads to cellular transformation and tumorigenesis. The v-src gene is expressed via a subgenomic mRNA, generated by splicing a leader from the 5' end of viral RNA to a site upstream from the v-src coding region. The splice donor site lies within the gag gene. We recently mapped the splice acceptor site to a position 75 nucleotides upstream from the v-src initiation codon. The gag gene initiation codon, present in the leader, is spliced in frame with the v-src coding region, but a termination codon lies between these two sites.

There are two reasons to believe that the splice acceptor site used to generate $mRNA^{v\text{-}src}$ is derived from the cellular src (c-src)gene. First, hybridization studies indicate that this region of the viral genome shares homology with c-src. Second, S1 mapping studies suggest that the homologous site in c-src is used as a splice acceptor site in generating $mRNA^{c\text{-}src}$.

The recombination event between viral and cellular sequences could occur with DNA, during RNA splicing, during reverse transcription, or as a combination of these mechanisms. These results indicate that viral and cellular sequences were originally joined in an intron within the 5' noncoding region of the c-src locus and that the splice acceptor site at the 3' end of that intron is used in the formation of $mRNA^{v\text{-}src}$. The presence within the viral genome of sequences from a cellular intron suggests that the initial joining of viral and cellular information occurred as a recombination event between viral and cellular DNA.

0661 DIFFERENTIAL EXPRESSION OF ENDOGENOUS TYPE C VIRAL GENES ISOLATED IN SOMATIC CELL HYBRIDS, Christine A. Kozak and Wallace P. Rowe, National Institutes of Health, Bethesda, MD 20205

Many mice carry chromosomal genes, V loci, for the expression of the ecotropic and xenotropic classes of type C viruses. We have examined virus inducibility patterns in hamster/mouse somatic cell hybrids in which the chromosomes bearing specific V loci were isolated from most, if not all, of the other mouse chromosomes. Hybrids which retained Bxv-1 (chromosome 1), the xenotropic locus of BALB mice, showed a time course of expression similar to that observed with embryo fibroblasts and BALB-3T3 cells. Virus production reached a peak 3-4 days after treatment with iododeoxyuridine (IUdR) and then sharply declined to trace levels by 8-10 days. These data suggest that not only virus production, but also the kinetics and magnitude of expression are determined either by Bxv-1 itself or a cis-acting regulator. Expression of the endogenous high ecotropic V locus of the AKR mouse, Akv-2 (chromosome 16), was studied in hybrids carrying only chromosome 16, or chromosome 16 and few other mouse chromosomes. Since these cells lacked the ecotropic cell surface receptor (chromosome 5), it was possible to examine the primary response of Akv-2 to induction in the absence of exogenous reinfection. Hybrids produced high titers of virus 2-4 days after induction with IUdR or 5-azacytidine. Virus levels declined only slightly thereafter and remained at high levels for periods in excess of 25 days. Thus, the activated high virus locus, Akv-2, is not subject to stringent negative control. Whether the different patterns of virus expression of Bxv-1 and Akv-2 are due to differences in the integrated viral genome, to chromosomal position effects, or to linked controlling elements remain to be determined.

0662 MULTIPLE ARRANGEMENTS OF VIRAL DNA AND AN ACTIVATED HOST ONCOGENE (C-MYC) IN BURSAL LYMPHOMAS, Gregory S. Payne, J. Michael Bishop, Harold E. Varmus, University of California, San Francisco, California 94143.
Proviruses of avian leukosis virus (ALV) are located in the vicinity of a putative cellular oncogene (c-myc) in ALV-induced bursal lymphomas. Enhanced expression of c-myc occurs in association with proviruses found in any of three configurations: (i) on the 5' side (upstream) of c-myc in the same transcriptional orientation; (ii) on the 3' side (downstream) of c-myc in the same orientation; and (iii) upstream, in the transcriptional orientation opposite to that of c-myc. Thus activation of adjacent cellular genes by retroviral DNA can involve mechanisms other than provision of a transcriptional promoter. To study these mechanisms further we are in the process of isolating recombinant bacteriophage carrying ALV proviruses linked to c-myc using tumor DNA exhibiting each configuration. We have introduced a recombinant molecule containing c-myc and an ALV provirus in configuration iii into chicken and mouse fibroblasts as a calcium phosphate precipitate in order to characterize it's transcriptional activity.

0663 ARRANGEMENT AND EXPRESSION OF ABELSON MURINE LEUKEMIA VIRUS RELATED NUCLEIC ACID SEQUENCES IN A NOVEL HUMAN pre-B LYMPHOBLASTIC LEUKEMIA. B. Dale, T. Wheeler, R.G. Smith, and B. Ozanne, Univ. Tx. Health Science Center at Dallas, Dallas, TX 75235.
Abelson murine leukemia virus (A-MuLV) can induce pre-B cell tumors in mice and transforms cells with a similar phenotype in tissue culture. The transforming gene of the virus is derived from nucleic acid sequences of Moloney murine leukemia virus (M-MuLV-specific sequences) and normal BALB/c mouse DNA (abl-specific sequences). The gene encodes a fusion protein, p120. Antisera against the abl-encoded sequences of p120 recognize a normal cell protein (NCP150) on mouse thymocytes and other lymphoid cells. NCP150 is the presumed gene product of endogenous abl sequences in the mouse. Sequences homologous to abl have been shown to be highly conserved in various species including man. Using a molecularly cloned nucleic acid probe specific for abl sequences, we have analyzed homologous DNA and RNA in a cell line derived from a novel pre-B lymphoblastic leukemia (SMS-SB)(J. Immunol. 126.596, 1981) and in other human cells. While all lymphoid tumors and normal human cells show homology to the abl probe, RNA from SMS-SB cells contains several unique high molecular weight species. Further, abl-related RNA in SMS-SB cells is present in greater quantities than in any other human cells studied. The abl-related DNA in SMS-SB cells is arranged in a fashion similar to that found in other human DNA, indicating that no gross rearrangement of genomic DNA has occurred. The expression of abl-related RNA in SMS-SB cells may be significant in that the phenotype of this human tumor cell line resembles that of A-MuLV-transformed mouse cells, particularly in the expression of intracellular mu chain.

0664 SEPARATION OF MULTIPLE PHOSPHOTYROSINE PHOSPHATASES FROM CHICKEN BRAIN, J.G.Foulkes, E. Erikson and R.L.Erikson, Department of Pathology, University of Colorado Health Sciences Center, Denver Colorado 80262.

Extensive genetic evidence has indicated that a single gene product of RNA tumor viruses is responsible for viral transformation of cells to the malignant state. Recently, the transforming gene products of a number of different viruses have been identified and found to be associated with a protein kinase activity. These enzymes show the novel property of phosphorylating tyrosine residues. Uninfected cells also contain protein kinases with specificity for tyrosine residues, suggesting the idea that these proteins may have a role in the control of normal cell growth. We therefore began to search for a protein phosphatase with activity towards phosphotyrosine, using chicken brain as a starting material and ^{32}P-labelled phosphotyrosine-casein as a substrate.

Fractionation of the brain extract by DEAE-cellulose chromatography and gel filtration revealed the presence of 2 major and 3 minor phosphotyrosine phosphatase activities. The activity which elutes in the DEAE breakthrough (20% of the total) is polydisperse on gel filtration, with a MW range of 60,000 - 30,000. The major activity (60% of the total) elutes from DEAE-cellulose at 145 mM NaCl, and has a MW of 43,000. The 3 minor activities have MWs of 210,000, 120,000 and 90,000. Data showing factors which regulate their activity, their activity towards other ^{32}P-tyrosine-labelled proteins, including pp60^{src}, and the relationship of these enzymes to the previously described phosphoserine phosphatases will be presented.

0665 RECOMBINANT MURINE LEUKEMIA VIRUSES IN AKR/J MICE, Winship Herr and Walter Gilbert, Biological Laboratories, Harvard University, Cambridge MA 02138.

The majority of AKR mice develope leukemia between six to nine months. Preleukemic mice, of five to six months, express recombinant retroviruses which carry sequences from the two classes of endogenous proviruses: ecotropic and xenotropic. We study the structure of retroviruses present in leukemic tissue with ecotropic and xenotropic specific hybridization probes from within and surrounding the recombinant region of two known recombinants: MCF 247 and MCF V1-36.

In the preleukemic thymus, beginning at four to five months, we observe a large amount of unintegrated recombinant virus in the thymus but not other tissues of AKR/J mice. Unintegrated virus is not present in leukemic tissue. Instead, large numbers, in some cases over fifteen, of recombinant viruses are integrated into the genome of the leukemic cell. The recombinant structures are characterized by xenotropic gp70 sequences and ecotropic amino terminal p15E sequences which most resemble the thymotropic and oncogenic MCF viruses. These proviruses are not present in the germ line of AKR/J mice and therefore reflect recombination between endogenous viruses during developement.

0666 CHARACTERIZATION OF REVERTANT CELL LINES WITH MULTIPLE INTEGRATED MOLONEY MURINE SARCOMA VIRUS GENOMES. Michael Graiser and Dino Dina, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

A Moloney murine sarcoma virus-transformed NRK cell line was found to contain three integrated viral genomes, all of which contain the v-mos gene. Two genomes are complete viral inserts while a third incomplete one is not expressed. One set of morphological revertants is characterized by a deletion which removes the majority of the v-mos gene in one of the inserts. These revertants still express v-mos transcripts equivalent in size to those of the parental line, in addition to a unique larger-sized transcript, possibly a precursor. Superinfection of revertants with murine leukemia virus (MuLV) results in the successful rescue of transforming MSV virus. MuLV may rescue a low-frequency mutant transforming viral insert. Replication through other cells after virus rescue will lead to retransformation above the observed low spontaneous level. MuLV could alternatively provide complementary functions necessary for processing of v-mos transcripts from a complete viral insert. MuLV rescue may be due to recombination events between incomplete viral genomes resulting in the production of new complete genomes with transforming capacity. To better characterize the v-mos sequences of the revertant lines, a genomic library of the parental transformant was cloned into lambda phage. The physical structure and transforming activities of these cloned v-mos sequences is being determined and this data will be used to identify and characterize those sequences retained and lost in revertants.

0667 CLONING OF A HUMAN ENDOGENOUS RETROVIRUS-LIKE SEQUENCE, Tom I. Bonner, Maurice Cohen and Catherine O'Connell, National Cancer Institute-FCRF, Frederick, MD 21701

We have screened a human DNA library using as probe a chimpanzee cellular DNA sequence which has distant homology to the polymerase and p15E genes of the endogenous baboon virus, M7. One set of human clones has restriction map and nucleotide sequence similarities which indicate that it has only about 2% nucleotide divergence relative to the chimpanzee sequence. The human clone is retrovirus-like in that there are regions which have DNA sequence homology to the p30, polymerase and p15E genes of Moloney leukemia virus (MoLV). Furthermore, the spacing between the points of homology are nearly identical to the corresponding spacings in MoLV. One stretch of the p30-like sequence when aligned with MoLV codes for 15 identical amino acids out of 23. A portion of the polymerase region similarly codes for an amino acid sequence which matches that of MoLV at 13 of 18 positions and of M7 at 15 of 18 positions. However, we judge that this clone will not form a replicating retrovirus since there appears to be an inframe termination condon 12 amino acids from the amino terminal of the p10 homolog. This retroviral sequence appears to have been stably integrated before the divergence of man and chimpanzee since the human and chimpanzee clones have 3-4 kilobases of identical 3' flanking sequence.

0668 CHARACTERIZATION OF GR-FeSV, A NEW FELINE SARCOMA VIRUS, M. Barbacid*, S. Rasheed†, K. Robbins*, S.A. Aaronson* and M.B. Gardner†. *Laboratory of Cellular and Molecular Biology, NCI, Bethesda, MD 20205. †Department of Pathology, University of Southern California, Los Angeles, CA 90003

A new strain of feline sarcoma virus has been isolated by Rasheed et al, (Virology, in press) from a spontaneous sarcoma of an eight-year-old domestic cat. This virus, designated GR-FeSV, induced sarcomas at high indicence after a short latent period in fetal and in newborn kittens. GR-FeSV, like known mammalian sarcoma viruses, has been found to be defective for replication but competent to transform fibroblasts in vitro. Non-producer transformed clones from several mammalian species have been obtained by infecting cells at limiting virus dilution. These cells have been utilized to demonstrate that the primary translational product of the GR-FeSV genome is a 70,000 dalton molecule that contains the amino-terminal protein of the FeLV gag gene precursor, p15, "fused" to a sarcoma virus specific polypeptide. GR-FeSV P70 is a heavily phosphorylated molecule that possesses an in vitro associated protein kinase activity capable of phosphorylating its own tyrosine residues as well as those of immune immunoglobulin G molecules. Current efforts at comparing the GR-FeSV transforming gene with those of known strains of feline sarcoma viruses as well as with other retroviruses whose gene products also exhibit associated tyrosine-specific protein kinase activity, will be presented.

0669 ISOLATED NATIVE PP60^{src} DOES NOT AUTOPHOSPHORYLATE AND INDUCES ACTIN TRANSFORMATION IN MICROINJECTED CELLS, Patricia F. Maness and Barcey J. Levy, Department of Biochemistry, School of Medicine, University of North Carolina, Chapel Hill, NC 27514

The src gene product of Rous sarcoma virus (pp60^{src}) has been isolated from the rat tumor cell line RR1022 in native form. pp60^{src} migrates as a single band of 62,000 daltons molecular weight on SDS gels under reducing conditions and is not proteolytically cleaved during isolation. Antipp60^{src} antibodies present in tumor bearing rabbit serum immunoprecipitate pp60^{src} from the purified material. Throughout the purification pp60^{src} is able to phosphorylate the heavy chain of antipp60^{src} immunoglobulin G in immunoprecipitates. On the basis of kinase activity in immunoprecipitates our isolation procedure results in a 7000 fold purification of pp60^{src} over detergent extracts. Isolated pp60^{src} is also able to function as a protein kinase outside the immunoprecipitate, phosphorylating antipp60^{src} antibody heavy chains and casein in solution. Histones (arginine and lysine-rich) and normal rabbit immunoglobulin G do not serve as phosphoacceptor substrates. Isolated pp60^{src} does not undergo autophosphorylation when incubated with ^{32}P-ATP-Mg, suggesting that cellular kinases carry out the phosphorylation of pp60^{src}. The availibility of purified native pp60^{src} provides a substrate for identifying these cellular kinases. The pp60^{src} isolated by this procedure is not only structurally native and an active protein kinase, but is also physiologically active, for it induces a dramatic rearrangement of actin cytoarchitecture, the "actin transformation", within 50 minutes following microinjection into normal 3T3 mouse fibroblasts.

0670 NATURE AND ORIGIN OF ACQUIRED CELLULAR GENES INVOLVED IN FeSV INDUCED TRANSFORMATION, Wim Van de Ven,[1] John Groffen,[2] Nora Heisterkamp,[2] Frank Grosveld[3] and John R. Stephenson,[2] [1]Frederick Cancer Research Facility, [2]National Cancer Institute, Frederick, MD 21701 and [3]Medical Research Center, Mill Hill, London, England NW71AA

The Gardner and Snyder-Theilen strains of feline sarcoma virus (FeSV) were derived by genetic recombination between cellular and viral genes. Both encode polyproteins of around 115,000 molecular weight (M_r) with tyrosine specific protein kinase activity. A second, widely-studied variant of Snyder-Theilen FeSV encodes a structurally-related gene product of only 85,000 M_r. To determine the relationship between the cellular transforming genes represented within these individual virus isolates, the integrated cellular forms of each were identified in nonproductively transformed cells, molecularly cloned in appropriate vector systems, and subjected to detailed restriction enzyme analysis. The results of these studies in combination with those of tryptic peptide analysis indicate that Gardner FeSV P115 differs from Snyder-Theilen FeSV P85 by an amino terminal region of approximately 20,000-30,000 M_r, while Snyder-Theilen FeSV P85 represents a truncated form of Snyder-Theilen FeSV P115. To directly test this model, in vitro recombinant genomes are being analyzed by transfection and by subcloning in specifically constructed expression plasmids. Additionally, the human cellular homologue of the Gardner/Snyder-Theilen FeSV acquired sequences has been molecularly cloned and its structural organization in relation to homologous human sequences, determined. The significance of these findings with respect to the origin of these independent transforming viruses will be discussed.

0671 HYBRIDOMAS SECRETING ANTIBODIES SPECIFIC TO TRANSFORMING POLYPROTEINS ENCODED BY INDEPENDENT ISOLATES OF FELINE SARCOMA VIRUS, Fulvia Veronese,[1] Gary J. Kelloff,[1] Fred H. Reynolds, Jr.,[2] Richard W. Hill[1] and John R. Stephenson,[1] [1]National Cancer Institute and [2]Frederick Cancer Research Facility, Frederick, MD 21701

To facilitate structural and functional analysis of transformation-specific polyprotein gene products of the Gardner, Snyder-Theilen and McDonough strains of feline sarcoma virus (FeSV), specific hybridomas were derived. Fisher rats were immunized by subcutaneous inoculation with nonproductively transformed syngeneic tumor cells, spleen cells harvested and fused to Lou rat Y3-Ag1.2.3. myeloma cells. Cellular proteins immunoprecipitated by culture fluids of resulting hybrids were initially screened by immunoprecipitation and further characterized on the basis of cell specificity and tryptic peptide composition. In addition to hybridomas producing antibodies against several as yet uncharacterized cellular phosphoproteins, hybridomas with antibody specificity for McDonough FeSV P170 and several clones recognizing antigenic determinants common to both the Gardner and Snyder-Theilen FeSV-encoded polyproteins were identified. High titered antibodies were obtained from ascitic fluids of Lou rats inoculated with each of the hybridomas. The inability to separate the Gardner and Snyder-Theilen FeSV polyproteins from their associated protein kinases by affinity chromatography utilizing these newly developed reagents argues that the enzymatic reactivities are intrinsic to the polyproteins. Under analogous purification procedures, McDonough FeSV P170 lacked detectable enzymatic activity. Studies are in progress to specifically localize antigenic determinants recognized by these antibodies and to explore their suitability for the identification of cellular analogues of FeSV encoded transforming proteins.

0672 THE RELATIONSHIP OF rel SEQUENCES IN RETICULOENDOTHELIOSIS VIRUS STRAIN T TO c-rel SEQUENCES IN NORMAL AVIAN DNAS, Irvin S. Y. Chen, Kirk C. Wilhelmson, and Howard M. Temin, McArdle Laboratory, University of Wisconsin, Madison, WI, 53706.

The transforming avian retrovirus, reticuloendotheliosis virus strain T (REV-T), contains sequences (v-rel) which are thought to be essential for transformation by the virus. Sequences homologous to v-rel can be detected in normal avian DNAs. These presumed progenitor sequences to v-rel are termed c-rel. Tofacilitate the study of the relation between v-rel and c-rel we obtained molecular clones of wild type REV-T DNA and molecular clones of c-rel sequences from line 15B chicken DNA.

The c-rel sequences appear to be present in a single copy per haploid genome. Approximately 1.0 kbp of the c-rel sequences are distributed over a region of about 20 kbp in chickens, including one large intervening region of 14 kbp. Clones of c-rel were also obtained from turkey DNA. These c-rel sequences also span a large region of DNA.

The expression of v-rel and c-rel was studied in REV-T infected cells and uninfected chicken tissue, respectively. Asubgenomic viral RNA of 2.5 kbp contains rel sequences in REV-T transformed cells. By contrast, two polyadenylated RNAs, 4.0 and 2.8 kbp, contain sequences homologous to rel in chicken spleen cells. The origin of these transcripts was mapped onto the regions of DNA containing c-rel.

0673 ESTABLISHMENT OF IN VITRO MYELOMONOCYTIC PERMANENT CELL LINES FROM IN VIVO VIRUS-INDUCED CHLOROLEUKEMIAS IN MICE. Serge Fichelson, Françoise Wendling, Jean-Michel Heard, Pierre Tambourin and Bruno Varet, Laboratoire Immunologie et Virologie des Tumeurs, INSERM U 152, Paris, Unité de Physiologie Cellulaire, INSERM U 22, Orsay, France.

Two clonal isolates of F-MuLV derived from the F. Lilly strain of FVP (643-22N and I^{-5}) induced leukemias of lymphocytic, erythroid or granulocytic origin within 20-35 weeks when inoculated to newborn DBA/2 or adult ICFW mice. Chloroleukemias were observed in 66% of newborn DBA/2 and 30% of adult ICFW. Both viruses were free of any detectable SFFV or MCF activity. Cells from chloroleukemic organs were serially transplantable intraperitoneally (i.p.) or subcutaneously (s.c.) into secondary syngeneic recipients. Six independent primary chloroleukemic cells were adapted to in vitro growth after inoculation to secondary recipients. In 3 cases, permanent cell lines were obtained in alpha medium with 10% fetal calf serum only. In 2 cases, the establishment of cell lines needed the addition of 10% WEHI-3B cells conditioned medium (CM). After several in vitro passages, the addition of CM was no more required. I.p. or s.c. inoculation of these culture cells led to the development of chloroleukemic tumors within 20-25 days in syngeneic mice. Cytochemical and functional properties of cultured cells were characteristic of myelomonocytic cells : strong positivity of myeloperoxydases, specific esterases, Fc receptor, ability to phagocyte latex particles. Clonal culture of these cells in agar suggested that they produced a factor necessary for their own growth. Since only few leukemic transplantable myelomonocytic cells growing in vitro are available, these 5 cell lines provide a new material to get some insight into the in vitro properties of granulocytic leukemias in mice.

0674 Characterization of a recovered MC29 with the ability to induce lymphoid tumours in vivo
G. Ramsay [1], L.N.Payne[2], T. Graf [3] and M. Hayman[1]

1. Imperial Cancer Research Fund, London,England. 2. Houghton Poultry Research Centre, Huntingdon, England. 3. Virusforchung, Deutsches Krebsforschungszentrum, Heidelberg, West Germany.

Avian myleocytomatosis virus transforms fibroblasts and macrophages in vitro while in vivo it causes a wide variety of tumours including myleocytomatosis, endotheliomas, kidney andliver carcinomas and sarcomas. We have isolated 3 td mutants of MC29 that have a reduced ability to transform macrophages and induce tumours whilst retaining their capacity to transform fibroblasts. One of these mutants, MC29 10H on passage through macrophages gave rise to a recovered virus MC29 HBI which in vitro transforms macrophages 100 times more efficiently than its parent while in vivo it produces a high incidence of lymphoid tumours. Analysis of HBI infected cells revealed a 108^{gagmyc} polyprotein as opposed to the $90K^{gagmyc}$ of td10H or the $110K^{gagmyc}$ of wt MC29. Tryptic peptide mapping demonstrated that the 108K protein had acquired v-myc specific peptides and phosphorylation sites absent from td 10H.90K but present in wt MC29-110K. These results suggest that td 10H has recombined with c-myc to generate a recovered MC29 virus which is capable of inducing lymphoid leukosis.

0675 ONCOGENES IN NATURALLY OCCURRING HUMAN TUMORS AND IN ESTABLISHED HUMAN TUMOR CELL LINES, S. Pulciani and M. Barbacid. Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland, 20205.

The presence of dominant oncogenes in naturally occurring human tumors has been investigated. High molecular weight DNAs from sixteen carcinomas and sarcomas were tested in tranfection assays. Four of these DNAs, including those isolated from pancreatic and colon carcinomas and from an embryonic rhabdomyosarcoma induced morphologic transformation in recipient mouse NIH/3T3 fibroblasts. Clonal transformants exhibited anchorage-independent growth and caused tumors when injected into athymic and immunocompetent mice. Moreover, they contained human DNA sequences, some of wich segregated with the transformed phenotype in subsequent cycles of transfection.

Transforming genetic elements have also been detected in two human tumor cell lines established from a fibrosarcoma and a bladder carcinoma. Biochemical analysis of their respective transforming sequences indicate that they have an independent genetic origin. Digestion of DNA from fibrosarcoma-transformed mouse cells with Eco RI, Hind III or Bam HI, completely abolished its transforming activity. In contrast, none of these enzymes affected transformation induced by the bladder carcinoma oncogene. Moreover, third cycle NIH/3T3 transformants derived from fibrosarcoma cells retained at least two copies of the Alu family of human repeated sequences. No such marker sequences were found to be part of the bladder carcinoma oncogene. These results support the concept that different oncogenes may be responsable for the onset of different types of neoplasia.

0676 EXPRESSION OF $pp60^{src}$ IN RSV-INFECTED MACROPHAGES, G. Steven Martin and Jerry C. Guyden, Dept. of Zoology, Univ. of California, Berkeley.

Chick macrophages can be transformed by the avian acute leukemia viruses MC29 and AMV but not by Rous sarcoma virus (RSV). However RSV-infected macrophages undergo a transient growth stimulation and exhibit enhanced uptake of 2-deoxyglucose. All of the cells in the culture become infected, as determined by immunofluorescent staining with anti-p19 serum. The intensity of fluorescence, and the quantity of gag polypeptides which can be immunoprecipitated from RSV-infected macrophages, are reduced when compared to RSV-infected fibroblasts. The quantity and activity of $pp60^{src}$ in RSV-infected macrophages is also reduced, as judged both by immunoprecipitation experiments using rabbit anti-tumor serum and by measurements of the phosphorylation of cellular polypeptides at tyrosine residues. These observations suggest that the failure of RSV-infected macrophages to undergo complete transformation may result from reduced levels of src expression. Tissue-specific controls on viral gene expression may explain other instances of target-cell specificity.

0677 THE AVIAN LEUKEMIA VIRUSES AMV AND E26 BOTH CONTAIN THE ONCOGENE myb BUT CAUSE DIFFERENT TYPES OF LEUKEMIA IN CHICKENS. Kathryn Radke, Hartmut Beug, and Thomas Graf. German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, West Germany.
Avian myeloblastosis virus (AMV) and E26 virus are acute leukemia viruses containing the oncogene, myb. AMV causes myeloid leukemia but E26 has been reported to cause erythroid or myeloid leukemia in chickens. We therefore identified the hematopoietic lineages of leukemia cells from chickens infected with RAV-2 pseudotypes of AMV or E26. E26-leukemic cells from the blood, bone marrow and spleen were identified as erythroid: they were predominately histone 5-positive, some were benzidine-positive, and they were a mixed population carrying surface antigens of immature, nearly mature, or mature erythroid cells. Myeloid AMV-leukemic cells, in contrast, were strongly positive for ATPase activity and carried a surface antigen of immature myeloid cells, but were negative for all erythroid markers. Only a few cells among E26-leukemic cells were positive for myeloid markers. E26-erythroid cells would not grow in vitro under conditions permitting the growth of avian erythroblastosis virus-erythroblasts or AMV-myeloblasts. Instead, E26-leukemic cells after a crisis gave rise to populations of proliferating myeloid cells that were indistinguishable from AMV-leukemic cells in culture. However, E26-erythroid cells in the peripheral blood included some immature erythroid cells with growth potential in vitro. Some of these cells grew into large, partially hemoglobinized colonies in methocel cultures supporting the growth of small, highly hemoglobinized CFU-E from normal bone marrow. Therefore, E26-infected chickens develop an erythroleukemia characterized by the presence of erythroid cells at various stages of maturity. Also present are a few infected myeloid cells which can grow in culture, but whose role in the leukemia is not yet clear.

0678 COMPARATIVE ANALYSIS OF THE ONCOGENES (fps) IN CLASS II AVIAN SARCOMA VIRUSES, Michael M.C. Lai, Timothy C. Wong, Akiko Hirano, and Peter K. Vogt, University of Southern California, School of Medicine, Los Angeles, CA 90033
Avian sarcoma viruses PRC II, PRC IV, PRC II-p and Fujinami sarcoma virus (FSV) have been shown to contain an oncogene (fps) of similar nature and therefore belong to the same group of sarcoma viruses. We have compared their genome structure by oligonucleotide fingerprinting and heteroduplex mapping. It was found that all of them contained various extents of deletion in the gap-pol-env genes and had inserted oncogenic sequences of different lengths in the middle of their genomes. PRC II-p and PRC IV have the largest genomes (6.1 kb). These two viruses have exactly identical fps genes (2.9 kb) as judged by nucleic acid hybridization and oligonucleotide fingerprinting. However, their replicative gene sequences are different. PRC II-p also retained a longer stretch of residual env gene. Therefore, these two viruses appear to have derived from recombination of different helper viruses with the same cellular fps gene. PRC II is completely homologous to PRC II-p except that the oncogene in PRC II is smaller (1.2 kb). The deletion corresponds to the 5'-half of the fps gene of PRC II-p.
FSV also has a small genome (4.7 kb) but contains all of the oncogenic sequences present in PRC IV. By combination of nucleic acid hybridization and oligonucleotide fingerprinting, we have shown that oncogenes of PRC IV and FSV differ mainly at the 5'-half of their respective oncogenes. Since all of these viruses have similar tyrosine-specific protein kinase activity and are oncogenic, we conclude that these activities reside in the 3'-half of their oncogenes. It is possible that the 5'-half sequences might potentiate the oncogenic activity.

0679 COMPLETE NUCLEOTIDE SEQUENCE OF THE TRANSFORMING GENE OF AVIAN MYELOBLASTOSIS VIRUS (AMV). T. S. Papas[1], K. E. Rushlow[1], J. A. Lautenberger[1], K. P. Samuel[1], D. K. Watson[1], N. C. Kan[1], T. W. Pryl[1], M. A. Baluda[2] and E. P. Reddy[3]. [1]Lab. of Molecular Oncology, [3]Lab. of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD; [2]UCLA School of Medicine, Molecular Biology Institute, Los Angeles, CA
AMV is a defective virus whose genome has undergone a sequence substitution, in which a portion of the region normally coding for the "env" protein has been replaced by cellular sequences. We have determined the complete nucleotide sequence of this region. Examination of the AMV oncogenic sequence revealed an open reading frame starting with the initiation codon ATG and terminating with the triplet TAG. The stretch of 795 nucleotides would code for a protein of 265 amino acids with a molecular weight of 30,000 daltons. The putative protein would contain 5 methionine residues while cyonogen bromide cleavage would produce four peptides whose sizes can be predicted by the location of initiation codons within the 265 amino acid sequences.

0680 INDUCTION OF 68K STRESS PROTEIN BY HEAT SHOCK, AD 5 INFECTION AND CERTAIN CHEMICALS
Rick Morimoto and Matthew Meselson, Harvard Biochemical Labs., Cambridge, MA

Brief exposure of mammalian cells to elevated temperature or certain forms of stress has a dramatic effect on gene expression. In human and mouse cells a 68K protein is induced by heat shock (41-43 C), a variety of chemicals that antagonize biosynthetic processes (arsenate, canavanine, $ZnCl_2$, $CuSO_4$, $CdSO_4$, disulfiram, FCCP, dbcAMP) and infection of human cells with Ad 5 virus. In an Ad 5 transformed human embryonic line 293, the major cellular protein is 68K. Exposure of 293 cells at 43 C for up to 4 hours has no apparent effect on protein synthesis except for the induction of 68K to higher levels. In other human cell lines (Hela, WI-38, WISH, HEK) a 1 hour heat shock substantially decreases the synthesis of all cellular proteins except for the 84K and 68K heat shock proteins. The function of the 68K protein is addressed by the observation that pre-shock at a non-lethal temperature (43 C) protects cells from subsequent exposure to a lethal temperature (45 C). Prior treatment of the cells with actinomycin D or cycloheximide blocks the protective effect, possibly by inhibiting the synthesis of 68K. Canavanine mimics the protective effect of a thermal pre-shock. The 84K protein is cytoplasmic before and after heat shock. In 293 cells at 37 C, 68K is primarily associated with the cytoplasm. During heat shock, 68K is partitioned 80:20 between cytoplasmic and nuclear fractions. Most of the 68K in the nuclear fraction becomes associated with the cytoplasmic fraction after return to 37 C for 4 hours. A subsequent heat shock reassociates 68K with the nuclear fraction.

0681 ASSOCIATION OF THE LETHAL YELLOW ($\underline{A^y}$) COAT COLOR MUTATION WITH AN ECOTROPIC MULV PROVIRUS, Neal G. Copeland, Nancy A. Jenkins and Barbara K. Lee, The Jackson Laboratory, Bar Harbor, ME 04609

The agouti locus of Chromosome 2 is a particularly interesting and complex locus of Mus musculus. The 17 alleles which have been described at this locus exceeds the number recorded for any other locus concerned with synthesis of melanin. Since an ecotropic provirus is associated with the dilute coat color mutation of DBA/2J mice, we were interested in determining if any coat color alleles of the agouti locus are also linked to ecotropic MuLV DNA sequences. Restriction analysis, Southern blotting and hybridization with an ecotropic MuLV DNA specific probe of DNAs from mice that collectively carried 10 different alleles at this locus, showed that one allele, the lethal yellow ($\underline{A^y}$) allele which is carried congenic and heterozygous on C57BL/6J, 129/Sv and LT/Sv mice, was closely linked to an ecotropic provirus. Littermates that did not carry the $\underline{A^y}$ allele did not carry this ecotropic provirus. This provirus was identical by restriction analysis to the AKR ecotropic provirus, was not spontaneously expressed in 129/Sv-$\underline{A^y}$ mice, but could be induced with IdU from 129/Sv-$\underline{A^y}$ kidney cells in vitro. In contrast, both YS/Icr-$\underline{A^y}$/a and YS/Icr-a/a mice carried the $\underline{A^y}$ associated ecotropic provirus. In addition, YBR/Ki-$\underline{A^y}$ did not carry the $\underline{A^y}$ associated provirus. However, they did carry two other proviruses which may be derived from the $\underline{A^y}$ associated provirus by recombination involving both 5' and 3' viral flanking cellular DNA sequences. Although a causal relationship between the presence of this provirus and the $\underline{A^y}$ mutation remains to be elucidated, the close linkage of this provirus to the agouti locus may be useful for cloning and characterizing the 17 alleles at this locus.

0682 BIOCHEMICAL CHARACTERIZATION OF TRANSFORMATION-SPECIFIC PROTEINS OF ACUTE AVIAN LEUKEMIA AND SARCOMA VIRUSES, Karin Moelling, Peter Donner, Masatoshi-Koji Owada, Irene Greiser-Wilke and Thomas Bunte, Max-Planck-Institut für Molekulare Genetik, D-1000 Berlin 33 (West).

The transformation-specific proteins of several avian oncornaviruses are expressed as fusion proteins containing parts of the viral structural gag-proteins. The N-terminal portion of the fusion proteins is represented by p19. Monoclonal antibodies were prepared against this protein and used for characterization of transforming fusion proteins. The monoclonal antibodies were applied for indirect immunofluorescence, immunoprecipitation of the proteins from subcellular fractions and for immuneaffinity column chromatography. The transforming proteins analyzed comprised the p110gag-myc, p130gag-fps and p75gag-erb. The gag precursor Pr75gag was used for comparison. The results obtained were: p110gag-myc is located in the cell nucleus of MC29-transformed fibroblasts, it is associated with a chromatin preparation and exhibits DNA-binding ability after 3000fold purification. p130gag-fps is present in nucleus and cytoplasm of FSV-transformed rat cells and is associated with a protein kinase activity after 2000fold purification which phosphorylates tyrosine and serine. The p75gag-erb is in the nucleus as well as in the cytoplasm, and after purification does not exhibit protein kinase activity nor DNA-binding ability. Pr76gag and the processed viral structural proteins are present in the cytoplasmic fraction to more than 90%, and after purification Pr76gag does not bind to DNA. Three deletion mutants of MC29 and a temperature-sensitive mutant of FSV were analyzed analogously. Furthermore, proteins were purified from fibroblasts as well as bone marrow target cells.

0683 TRANSFORMATION BY A LUNG CARCINOMA INDUCING VIRUS, U. R. Rapp, A. Rizzino, E. Birkenmeier and T. Bonner, National Cancer Institute, Frederick, MD 21701

We have previously reported the isolation of a lung adenocarcinoma inducing mouse retrovirus by selection from a population of IdUrd induced C3H/MuLV (PNAS, 77: 624-628, 1980). Here we describe (1) a characterization of its target cell in vivo and (2) the biological activity of genomic viral DNA that was obtained by molecular cloning of unintegrated provirus from virus transformed Mv-1Lu cells. 1) The target cell was characterized by the derivation of cell lines from virus induced lung adenocarcinomas. These cells form adenocarcinomas when injected into syngeneic mice and in culture retain properties typical of alveologenic type 1 cells. These include morphological details, as well as the ability to transport fluid in culture. Fluid transport becomes evident by the formation of domes or hemicysts. The formation of domes, which is considered to reflect a differentiated function of these secretory epithelial cells, can be enhanced more than 50-fold by treatment with dibutyryl cyclic AMP.
2) Infection of Mv-1Lu cells in culture with lung carcinoma virus induces these cells to grow in soft agar (SA). Clonal derivatives from such SA colonies are sensitized toward the action of epidermal growth factor (EGF). Thus, addition of EGF at 10 ng/ml to soft agar reversibly increases both the rate of growth (3-fold) as well as the fraction of the cells (5-fold) forming colonies in SA. Mv-1Lu cells that were infected with virus obtained by transfection of cloned viral DNA also respond to treatment with EGF. Uninfected Mv-1Lu cells do not show this effect. These findings suggest that the mechanism of transformation by the lung carcinoma virus may involve the sensitization of alveologenic lung cells to the action of EGF.

0684 ISOLATION OF NEW MAMMALIAN TYPE C TRANSFORMING VIRUSES, U.R. Rapp, F.H. Reynolds, Jr. and J.R. Stephenson, National Cancer Institute-FCRF, Frederick, MD 21701

Newly-isolated acute transforming virus of mouse origin, designated 3611- and 2207-MuLV, respectively, transform embryo fibroblasts and epithelial cells in culture. Mice inoculated at birth with either virus develop tumors within four weeks; these contain several distinct mesenchymal cell types with histiocytes and fibroblasts as the predominant component. These two new virus isolates resemble previously described mammalian acute transforming viruses in that both are replication-defective, requiring a type C helper virus for successful propagation both in vitro and in vivo. By endpoint transmission of either virus to mouse or rat cells, several nonproductively transformed clones have been isolated. Pseudotype virus stocks obtained from such clones transform cells in vitro, are highly oncogenic in vivo, and exhibit host range and serologic properties characteristic of the helper virus. Although 2207-MuLV transformed cells lack detectable levels of viral antigen expression, analysis of 3611-MuLV transformed clones led to the demonstration of a 100,000 molecular weight (M_r) polyprotein and an 85,000 M_r possible cleavage product consisting of amino terminal MuLV gag gene proteins, p15 and p12, linked to an acquired sequence encoded nonstructural component. In contrast to the 120,000 M_r polyprotein translational product of the Abelson strain of MuLV, the 3611-MuLV major gene product was not subject to autophosphorylation in vitro and lacked detectable tyrosine phosphorylation in vivo. The relatedness of these newly-isolated transforming virus isolates to previously described mammalian transforming viruses will be discussed.

0685 MONOCLONAL ANTIBODIES TO ANTIGENIC DETERMINANTS ENCODED BY THE VIRAL ONCOGENE (v-fms) OF MCDONOUGH FELINE SARCOMA VIRUS, Soni Jo Anderson, Mark Furth, Linda Wolff, Sandra Ruscetti, and Charles J. Sherr, Laboratory of Tumor Virus Genetics, National Cancer Institute, NIH, Bethesda, MD 20205.

The provirus of the McDonough strain of feline sarcoma virus (SM-FeSV) [gene order: 5'-gag-fms-env-3'] contains a 3.0 ± 0.3 kb viral oncogene (v-fms) which shows no homology to the transforming gene (v-fes) of other characterized FeSV strains (Donner et al, J. Virol. 41, 489-500, 1982). The gag and fms sequences encode a 180 kd fusion polyprotein ($P180^{gag-fms}$) which is cleaved to two products, $P120^{fms}$ and $pp60^{gag}$, in lysates of SM-FeSV transformed cells. Both P180 and P120 are glycosylated, highly membrane-associated, and lack phosphotyrosine residues in vivo, suggesting that the SM-FeSV gene product differs functionally from the tyrosine kinases associated with v-fes coded products. Monoclonal antibodies specific for antigenic determinants encoded by v-fms have now been prepared by immunizing rats with live, syngeneic SM-FeSV transformed NRK cells (Ruscetti et al, J. Virol. 35, 259-264, 1980) and fusing splenic lymphocytes from tumor-bearing animals with Y3-Ag1.2.3 rat myeloma cells (Galfre et al, Nature 277, 131-133, 1979). Lymphocyte-myeloma hybrids producing antibodies to P180 and P120 were identified by precipitation of labeled polypeptides from SM-FeSV transformed mink cells. Four positive hybrids were cloned twice in soft agar, established as stable lines, and grown in defined serum-free medium to facilitate purification of homogeneous antibodies to v-fms determinants. These reagents should enable the localization of transforming gene products in SM-FeSV transformants and provide a means to detect products encoded by the homologous cellular gene (c-fms).

Poster Session IV

0686 Construction of vectors from the genome of MoMuLV for the expression of nonselectable genes in mammalian cells; Eli Gilboa, J. S. Park, S. Hwang, R. Kucherlapati, K. Noonan, H. Greeman and M. Kolbe.

We have functionally identified and physically isolated viral DNA fragments required for the expression of the viral gag/pol and envelope coding sequences. Using several procedures, we have generated DNA fragments from the genome of MoMuLV, enzymatically joined to DNA fragments carrying the coding sequences of Herpes TK, mouse DHFR and bacterial neo^r genes; transfected the hybrid DNA into mouse cells and followed their expression.

Using this information we have constructed a set of versatile vectors for the expression nonselectable genes (including cDNA copies of mRNAs) by effectively replacing the coding sequences of one of the viral genes with a selectable marker like Herpes TK and replacing the second gene with other nonselectable cDNA fragments.

In vitro reconstructed genes are used to study the mechanism of mammalian gene expression. The use of selectable vectors enables the isolation and analysis of cells carrying a defective gene construct.

0687 RESIDUAL TRANSFORMATION BY TRANSFORMATION DEFECTIVE MUTANTS OF POLYOMA VIRUS Michele M. Fluck, Microbiology, Michigan State University, East Lansing, MI,48824

Hr-t mutants of polyoma virus have been reported to be transformation defective. However, surprisingly we often observe that when monolayers of Fisher rat cells are infected and kept for extremely long times (6 - 10 weeks), small, rare and delayed foci appear in the hr-t infected cultures. We are studying the possibility that these represent viral transformants of a rare kind. The presence of viral sequences, the expression of viral genes, and the transformation phenotype of such clones are under study.

0688 DIFFERENTIATION AND ACTIVATION OF SPLENIC T CELL PRECURSORS BY MATURE PERIPHERAL T CELLS IN THE ABSENCE OF THYMUS, Joseph S. Lipsick and Nathan O. Kaplan, UC San Diego, La Jolla, California 92093

Nu/nu splenic T cell precursors lacking significant Thy-1 surface antigen are driven in vitro to proliferate and express Thy-1 during incubation with activated, non-dividing peripheral T cells in the absence of thymus or thymic extracts. The precursors are present in nu/+ spleen as well, and are phenotypically similar to thymocyte precursors assayed in vivo. The non-dividing inducer cells required are Lyt-2^-, Thy-1^+ T cells present in nu/+ but not nu/nu spleen and show no MHC-restriction in the induction process. Using this in vitro assay we have preliminarily identified a monoclonal rat anti-mouse brain antibody which lyses nu/nu responding T cell precursors in the presence of complement. This monoclonal antibody has previously been shown to recognize the bone marrow target cell for Abelson murine leukemia virus transformation, but not CFU-s cells in bone marrow (Shinefeld, et al., CELL 20, 11, 1980). It also does not appear to recognize mature peripheral T cells responsive to Con A, although it reacts with most thymocytes.

The ability of mature peripheral T cells to induce the differentiation and activation of T cell precursors in the complete absence of thymus appears to explain the grossly different effects of neonatal and adult thymectomy. The in vitro assay described here for demonstrating the differentiation and activation of these precursors will allow further phenotypic characterization of these precursors and their susceptibility to infection and transformation by murine leukemia viruses.

0689 CONSTRUCTION OF A FUNCTIONAL HUMAN SUPPRESSOR tRNA GENE, G.F. Temple, Bethesda Research Laboratories, Inc., Gaithersburg, MD 20877; A.Dozy and Y.W. Kan, University of California, San Francisco, San Francisco, CA 94143

We have created a functional amber suppressor tRNA gene by site-directed in vitro mutagenesis. We began with a human $tRNA^{Lys}_{AAA}$ gene, and subcloned this into M13.MP7. With the aid of a synthetic 15 nucleotide primer which had two non-complementary nucleotides to the anticodon region of this gene, we converted the codon recognition of this gene from AAA to UAG. This was accomplished by extending the primer with DNA ploymerase and making closed circular double stranded DNA in the presence of T_4DNA ligase. This DNA was used to transform E. coli to yield mutated and unmutated M13-recombinants. Successive rounds of synthesis and purification permitted us to isolate recombinant phage, 100% of which were mutated to recognize the UAG codon.

The mutated tRNA gene was shown to actively direct transcription of tRNA in vivo in the frog oocyte. This tRNA functioned as an UAG suppressor tRNA as demonstrated by its ability to suppress the UAG mutation in certain Chinese β^0 thalassemia mRNA, allowing synthesis of normal β globin.

Using the same procedure, we have recently converted a human $tRNA^{Gln}_{CAG}$ to $tRNA^{Gln}_{UAG}$ and are characterizing its activity in suppressing the nonsense mutation in certain Mediterranean β^0 thalassemia mRNA. Before introduction of suppressor tRNAs can be considered as a method of gene therapy, their effects on other cell functions must be assessed and is under investigation.

0690 FATE OF TRANSFECTED SEQUENCE VARIANTS OF SV40 AND MAMMALIAN DNA SEQUENCES, R. F. Baker, M. T. Harrigan, and G. Edelman, Molecular Biology Division, University of Southern California, Los Angeles, CA 90007.

Early gene regions of SV40 DNA, amplified on PBR322 in E. coli, have been transfected into mouse 3T3 cells and primary rat embryo cells. Selection for a transformed phenotype at low serum concentration yields many cells that form foci and fewer cells that form colonies in soft agar. Bgl II and Sst I restricted chromosomal DNA pieces from heterogenous populations and homogeneous subclones of the colony-forming cells were compared by Southern gel analysis. Individual cellular DNA restriction pieces that have SV40 and/or PBR322 DNA sequences attached have also been amplified in E. coli and these sequences have been analyzed separately. In all of these experiments, and in contrast to integration of wild type SV40 DNA into mouse cells, there is evidence of non-randomness of covalent linkage of the recombinant SV40 sequence into cellular DNA sites.

Subclones of 3T3 cells, transformed as a result of transfection by human placental DNA, have yielded supercoiled DNA from Hirt supernatant if the 3T3 cells are, after transfection, subjected to three or more rounds of: phenotype selection, subcloning, Hirt extraction, and finally retransfection of fresh 3T3 cells with the DNA from the Hirt supernatant. We are attempting to use this finding of supercoiled DNA as a method for obtaining (1) a rescued oncogenic sequence on a recovered (Hirt) plasmid, and (2) a plasmid sequence which allows equal distribution of replicated plasmids into daughter cells (i.e., a centromere-like DNA sequence).

0691 MOLECULAR ANALYSIS OF MOUSE ERYTHROID DEVELOPMENT: USE OF NON-GLOBIN RECOMBINANT DNA PROBES, P. Goldfarb, N. Affara, P. Harrison and J. O'Prey, The Beatson Institute for Cancer Research, Glasgow, G61 1BD, Scotland, U.K.

cDNA libraries from the total mRNA of mouse reticulocytes, fetal liver and Friend cells have been cloned in pAT 153 and screened for erythroid-specific non-globin recombinants. Similarly a mouse genomic library has been differentially screened with cDNAs from fetal liver and mouse embryo fibroblast cells to identify recombinants carrying erythroid genes. The cDNA plasmid clones are being used to study the transcription and DNAase I sensitivity of erythroid sequences in hemopoietic tissues and Friend cells. The clones are also being used to isolate and characterise their genomic equivalents. Genomic DNA recombinants from a λ Ch4A library have been analysed at the structural level to identify erythroid coding sequences, and by selection and translation of mRNA from normal and Friend virus transformed cells to examine gene expression. The erythroid-specific recombinants will enable us to study the structure, expression and regulation of a series of non-globin erythroid genes in normal and leukemic cells.

0692 TRANSFORMATION OF MOUSE - AND RAT-FIBROBLASTS BY BOVINE PAPILLOMA VIRUS DNA HYBRID MOLECULES, Hans-Ulrich Bernard, Robert Brown and Günter Schütz, German Cancer Research Center, Heidelberg.

The genome of bovine papilloma virus (BPVI) can exist as high copy number, circular DNA molecule in bovine warts, in tumors and in in vitro cultured cells of various mammalian species. - Different hybrid molecules have been constructed that contain all or part of this viral DNA, the HSV or chicken tk genes and E. coli plasmid sequences. These DNA molecules can be selected and amplified in E. coli, and they contain a functional replicator and selector for mammalian cells. DNA mediated gene transfer into mouse 3T3 tk^- and Rat 2 tk cells cells allowed the selection of HAT-resistant tk^+ clones. These clones did arise with the same frequency as in cells being treated with tk plasmids not carrying the viral replicator. HAT-resistant clones with phenotypically transformed and untransformed phenotype occurred with similar frequency. Southern blot analysis of the DNA of these clones showed all transferred molecules to occur in rearranged structures in 1 or few copies per cell. Many of these structures were compatible with an intrachromosomal state of the BPV-hybrid-DNA. - These data show that extrachromosomal maintenance and high copy number are no necessary prerequisits for malignant transformation of fibroblasts by BPVI. Experiments are in progress to determine the limitations for extrachromosomal maintenance of BPV-derived DNA molecules in mammalian tissue cultures.

0693 PERMANENT EXPRESSION OF EARLY AND LATE FUNCTIONS OF POLYOMA E.C. MUTANTS IN F9 CELLS. Marc Vasseur,Génétique cellulaire,Institut Pasteur,Paris. Polyoma virus can develop lytically in differentiated mouse cells, but is unable to express either early or late functions in undifferentiated early embryonic cells or in embryonal carcinoma (E.C.) cells of the mouse. We have isolated polyoma virus mutants able to develop normally in E.C. cells. However,E.C. cells infection with py.E.C. mutants is productivebut not lytic, and permanently infected cells can be propagated indefinitly. We have isolated clones from an F9 cell line infected with py. E.C. mutants. In these clones, the percentage of cells expressing early and late viral proteins varies from 10 to 100%.The presence of multiplying virus in these cells do not affect the cloning efficiency and the growth rate. However,all the clones obtained so far share a common morphological modification:the cells are flat and do not aggregate to each other;electron microscopy observation reveal the presence of desmosomes and Golgi apparatus structuration.Other analysis (cytosquelletton,cell surface antigens) support the hypothesis that the permanent viral expression in the E.C. cells have induced an early and partial differentiation process.

0694 HOST-RANGE MUTANTS OF POLYOMA ARE AFFECTED IN VIRION MATURATION, Robert L. Garcea*[#] and Thomas L. Benjamin[#], Division of Pediatric Hematology, Children's Hospital Medical Center and Sidney Farber Cancer Institute*, and Dept. of Pathology, Harvard Medical School[#], Boston, Massachusetts 02115

We have studied the host-range transforming (hr-t) mutants of polyoma to investigate the defect in virus production of these mutants when infecting "non-permissive" mouse cells. When hr-t mutants infect NIH 3T3 mouse fibroblasts, the output of infectious virus is 20-100 fold lower than that of a corresponding wild type (wt) infection, whereas infection of permissive baby mouse kidney (BMK) cells yields a comparable output to wt. We have determined that hr-t infection of 3T3 cells yields ∿30% of the viral DNA and 75-100% of the capsid protein VP_1 as produced in the wt infection. We have developed a method for isolating polyoma viral maturation intermediates which reveals an apparent block in the maturation of the 75 S minichromosome to 240 S virion in the hr-t infection of 3T3 but not BMK cells. Two-dimensional gel analysis of total lysates from infected cells shows that the modified forms of VP_1 are dramatically reduced in hr-t infections. However, intact wt or hr-t virions contain equivalent amounts of each VP_1 subspecies. We propose that 1) the hr-t gene products control directly or indirectly VP_1 modifications which are necessary for virion assembly, 2) a reduction in the amounts of VP_1 subspecies leads to a decreased virus output (since all VP_1 forms are required for intact virions), and 3) permissive cells partially overcome this modification block leading to near normal virus outputs.

0695 INTEGRATION AND EXPRESSION OF A TRUNCATED SV40 EARLY GENE FRAGMENT IN MAMMALIAN CELLS. Wolfgang Deppert[1], Christine Weckler[2], Elard Jacob[3] and Jürgen Horst[2], University of Ulm, [1]Department of Biochemistry and [2]Department of Human Genetics, D 7900 Ulm; [3]Max-Planck-Institute for Virus Research, D 7400 Tübingen, F.R.G.

A recombinant plasmid based on pBR322 has been constructed which carries about 50 percent of the replicator proximal early region of SV40 DNA, including the viral origin of replication. It thus includes all coding sequences for SV40 small t-antigen, but lacks a major part of the SV40 large T-antigen (T-Ag) 3'-coding region. The plasmid was transferred together with herpes simplex virus (HSV) thymidine kinase (TK) gene as a selectable marker to mouse LTK^- cells. TK^+ cell clones were isolated and their high molecular weight (hmw) DNAs were shown by DNA blotting and hybridization experiments to contain the SV40 DNA fragment of the recombinant. In some of these clones a heterogeneous expression of the SV40 DNA fragment could be detected by immunofluorescence microscopy. Positive cells showed a nuclear fluorescence indistinguishable from that of SV40 wild type transformants, indicating that less than the NH_2-terminal half of T-Ag is sufficient for production of a T-Ag like fluorescence. Immunoprecipitation of labeled extracts of fluorescence positive transformants identified a T-Ag fragment with a M_r of 35 Kd. This molecular weight corresponded well to the expected coding capacity of the SV40 DNA in the recombinant plasmid. Surprisingly, however, small t-antigen could not be detected. Our results demonstrate that a truncated fragment of the early SV40 DNA can be stably integrated into mammalian cells, and that the SV40 information is expressed in some of these cells. This provides a tool for analyzing potential functions of NH_2-terminal fragments of T-Ag.

0696 EVIDENCE FOR AN SV40 LATE TRANSCRIPTIONAL CONTROL FACTOR: MIXED INFECTION OF A LATE LEADER DELETION MUTANT AND WILD TYPE EXHIBIT A TRANS EFFECT ON LATE TRANSCRIPTION.
James C. Alwine, University of Pennsylvania, Philadelphia, Pa., 19104
Mixed infections involving equal mutliplicities of wild type Simian Virus 40 (SV40) and viable deletion mutant d1861, in comparison to individual infections, result in decreased cytoplasmic levels of wild type derived late mRNA as well as very low to undetectable levels of mutant derived late mRNA. The d1861 deletion removes 16-25 base pairs from the late leader region. This deletion was shown to be the direct cause of the mixed infection effect; replacement of the deletion with wild type sequences restored normal levels of late mRNAs in mixed infections. Other viral functions, e.g. early gene expression and replication, were found to be uneffected by the d1861 deletion. Further examination of the mixed infection effect showed that the levels of unspliced, nuclear precursors of late mRNA, derived from each genome, were decreased or undetectable in accord with the cytoplasmic results. Thus the effect appears to be occuring at the transcriptional level. Overall the data demonstrated a *trans acting effect on late transcription*, which is detectable due to the presence of the d1861 mutant in the mixed infection. This finding is indicative of a diffusable factor which exerts a control on SV40 late gene expression at the transcriptional level. Based on the data a positive control model can be postulated. 11/11/81.

0697 MEMBRANE ANTIGEN EXPRESSION ON DIFFERENTIATING ERYTHROID CELLS AND ON PRE- AND POST-CHEMICALLY INDUCED DIFFERENTIATING AVIAN ERYTHROBLASTOSIS VIRUS-TRANSFORMED ERYTHROID CELLS. Bob G. Sanders, Kimberly Kline, Carrie H. Nelson and James P. Allison, University of Texas, Austin, Texas, 78712; and University of Texas System Cancer Center, Science Park Research Division, Smithville, Texas, 78757.
Monoclonal and monospecific reacting polyclonal antibodies specific for chicken hemopoietic-lymphoid cells detect membrane antigens that are differentially expressed on the erythroid cells of the developing chicken and on cloned avian erythroblastosis virus (AEV)-transformed erythroid cells before and after chemically induced cellular differentiation. Monoclonal and polyclonal antibodies to chicken fetal antigen (CFA) reveal a minimum of 13 serologically distinct antigens that are immunoprecipitated as a molecule with an apparent molecular weight of 50Kd. Antisera to chicken adult antigen (CAA) reveal a minimum of 7 serologically distinct antigens that are immunoprecipitated as molecules with apparent molecular weights of 45Kd and 84Kd. Three distinct definitive erythroid populations are detectable in the bone marrow and peripheral blood of the developing chicken based on their CFA and CAA expression. Prior to butyric acid induced maturation, cloned erythroleukemia cells exhibit neither globin nor hemoglobin but do express a major 84Kd and a minor 45Kd molecule detected by antisera to CAA and a 50Kd membrane associated molecule detected by antisera to CFA. Three days following butyric acid treatment, the cloned erythroleukemia cells expressed both alpha globin and hemoglobin. Analysis of membrane molecules on induced cells showed major changes with a major decrease in the 84Kd molecule, a major increase in the 45Kd molecule, and a reduction and slight MW shift in the 50Kd molecule. NIH Grant CA 12851, BGS; & CA 26321 & CA 26981, JPA.

0698 SPLICING OF INTRONS DURING PASSAGE IN A RETROVIRUS VECTOR, Joe Sorge and Stephen H. Hughes, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724
The gene for the coding region of alpha human chorionic gonadotropin (2700 b.p.) was inserted into cloned avian sarcoma virus DNA in place of the v-src gene (1700 b.p.), which had been deleted by in vitro manipulations. The recombinant viral DNA was not infectious by transfection. By in vitro manipulations, 1000 b.p. were removed from the center of one of the introns of HCG and this smaller variant was transfected into normal chicken fibroblasts. Virus grew in high titers and was passed to uninfected chicken fibroblasts and allowed to grow for 3 weeks. Examination of total cellular RNA by S1 nuclease mapping revealed that the HCG introns had been spliced precisely from about two thirds of the pooled viral RNA, whereas one third had retained the introns. One interpretation is that a subpopulation of viral RNA, destined to be packaged into virions, is protected from splicing; whereas RNA destined for protein synthesis is spliced normally. Presumably, the construction containing the 2700 b.p. insert did not grow due to a size constraint that could have been eliminated had the cells been able to splice the introns from genomic viral RNA. These results question the hypothesis that proto-oncogenes might be "captured" at the DNA level and converted to intron-less viral oncogenes.

0699 SPECIFIC GENE TRANSFER DURING WHOLE CELL FUSION OF HUMAN CELLS, Eric Stanbridge, Bernard Weissman, Corey Marks and William Benedict, University of California, Irvine, CA 92717 and University of Southern California, School of Medicine, Los Angeles, CA 90033

When human fibrosarcoma HT1080 cells were fused with HPRT$^-$ ouabain resistant normal human fibroblasts numerous colonies arose in the selective medium of HAT and ouabain. Chromosomal and biochemical analysis revealed that two classes of clones existed: Class I represented true hybrids and Class II was transformants of HT1080 that had acquired the ouabain resistant phenotype. Biochemical data showed that unselected markers from the fibroblasts were not transferred to the Class II HT1080 transformants. Furthermore, detailed chromosome analysis showed that the transformants contained only HT1080 chromosomes. Finally, stability studies of ouabain resistance showed that most of the Class II transformants were unstable for this phenotype suggesting a non-integrated state of the gene(s) encoding ouabain resistance. These studies show that specific gene transfer may occur during whole cell fusion of human cells.

0700 THE ROLE OF 3'-END SIGNALS IN GENE EXPRESSION. Charles N. Cole, George Santangelo, and Joanne Tornow, Department of Human Genetics, Yale University, New Haven, CT. 06510

The vast majority of genes transcribed by RNA polymerase II which have been studied contain signals at their 3'-ends which are involved in processing and polyadenylation of the mRNA. These signals appear to include the sequence 5'-AAUAAA-3' but this alone is not sufficient to cause processing and polyadenylation. In addition, many mRNAs contain the sequence 5'-TTTTCACTGC-3' at the poly A junction or, in the case of some histone mRNAs, near the 3'-end. The role of this sequence signal remains a mystery. Some histone genes, and genes for small nuclear RNAs, produce RNA products which lack poly A sequences but which nonetheless possess discrete 3'-ends. The nature of the signals involved in their 3'-end maturation are unknown. Finally, whether true transcription termination occurs in the transcription of genes by RNA polymerase II is not known. We have been investigating the role of 3'-end signals in gene expression. Deletion of these signals from either the herpes simplex virus thymidine kinase gene, carried on a plasmid capable of replication in cos-1 cells, or from SV40, results in the absence of gene expression. Initiation of transcription appears to be normal in both cases, but gene product is not produced at detectable levels in either case. The precise architecture of the transcripts produced is under investigation. Experiments are also in progress which involve the insertion of putative 3'-end regulatory signals from both viral (SV40, polyoma) and non-viral (B-globin, histone, small nuclear RNA U1) sources into the tk gene construct in attempts to define the type of 3'-end signals which can be used to allow maturation of the 3'-ends of the tk mRNA and its subsequent expression.

0701 STRUCTURAL ANALYSIS OF GENES CODING FOR HISTOCOMPATIBILITY ANTIGENS, A. Frischauf, S. Kvist, R. Buckland, H. Delius, D.P. Leader, H. Lehrach and B. Dobberstein, European Molecular Biology Laboratory, D-69 Heidelberg, Postfach 102209, Germany

A genomic library of DBA/2 mouse DNA (d haplotype) was constructed. More than 20 independent phages were isolated by hybridization with H-2 specific cDNA clones (Kvist et al., Proc. Natl. Acad. Sci. 1981, 78, 2772). Several genomic clones were characterized by restriction mapping and Southern blotting, and in addition by electron microscopy of heteroduplexes of different phages, hybrids of phages and cDNA sequences cloned in M13, and hybrids of phages and mRNA. Partial DNA sequence analysis was carried out showing the correspondence between protein domains and exon patterns. Clone VIII-3 was characterized in detail and it was found that it is very homologous (approx. 90%) to the characterized cDNA clones in the coding sequences. It does not, however, correspond to the K, D or L locus gene. The relationship between various clones will be discussed in terms of sequence homologies and possible expression of genes not unambiguously assigned to characterized expressed proteins.
A method for rapid restriction mapping of λ clones will be described.

0703 INTEGRATION AND EXPRESSION OF FV COMPLEX IN DIFFERENTIATED AND TRANSFORMED CELLS. R.Kollek, W.Ostertag, D.Frisby(1), G.Colletta(1), J.Greenberger(2) and N.Kluge(3). Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, West-Germany. (1)Glasgow, (2)Boston, (3)Hamburg

SFFV and MuLV-F of the Friend virus complex (FV) have multiple nonidentical integration sites in Friend cells (FLC). About 10-20 copies are integrated. Some of the integrated copies appear to be altered and are diagnostic for different Friend cell clones, some other Friend virus related (and altered) provirus appear to be common to most, if not all, FLC. Myeloid and stem cell lines isolated on infection of DEXTER type cultures with FV are heterogenous: all cell lines studied up to now show integration of MuLV-F, usually in low copy number. In most of these cell lines SFFV is not present. One exceptional permanent myeloid cell clone has several copies of both SFFV and MuLV-F and also altered sequences similarly as found in FLC. Expression of SFFV and MuLV-F related RNA shows a high level of expression of SFFV RNA in FLC but not in other transformed hematopoietic cell lines where MuLV-F is expressed at different levels. Expression in FLC x fibroblast, FLC x hepatoma and FLC x teratocarcinoma cell hybrids of non FLC phenotype is either decreased (fibroblast, hepatoma phenotype hybrids) or entirely absent (teratocarcinoma phenotype hybrids). Expression of SFFV and MuLV-F in these cells is compared to that of leukemic cells of mice infected by FV in vitro.

0704 REGULATION OF eIF-2 PHOSPHATASE ACTIVITY BY THE FUNCTIONAL STATE OF eIF-2, Brian Safer Deborah Crouch, Andrzej Konieczny and Rosemary Jagus, NHLBI, NIH, Bethesda, MD 20205

Phosphorylation/dephosphorylation mechanisms can produce highly specific changes of metabolic function in vivo. Purified protein kinases and phosphatases, however, frequently show a relative loss of specificity in vitro. We have examined the regulation of the phosphorylation state of eIF-2 as a model system to study the basis of this differential behavior. In unfractionated reticulocyte lysate, the phosphorylation state of eIF-2α can be rapidly altered, while phosphorylation sites of eIF-2β appear to be metabolically stable (Safer and Jagus, PNAS 76 1094, 1979). Purified eIF-2 phosphatase, however, rapidly dephosphorylates both the α and β subunits of eIF-2 (Crouch and Safer, JBC 255, 7918, 1980). This alteration of eIF-2 phosphatase specificity may result from the association of eIF-2 with a 394,000 dalton polypeptide complex in lysate which we designate RF. Formation of the RF·eIF-2 complex appears to affect both kinase and phosphatase accessibility to phosphorylation sites on the eIF-2α and β subunits and the 67,000 dalton subunit of RF. During protein synthesis initiation, there is a functional dissociation of the RF·eIF-2 complex during binding of the eIF-2·GTP·Met-tRNA$_i$ ternary complex to initiating 43S ribosomal subunits. Following the release of eIF-2 from completed 80S initiation complexes, reassociation of RF and eIF-2, required for eIF-2 reutilization, may be regulated by rapid changes in their phosphorylation and/or oxidation/reduction states of cysteine residues. Altered eIF-2 phosphatase specificity may therefore reflect, as well as effect, changes in the eIF-2 activity cycle through modulation of its interactions with RF.

0705 THE CHOICE BETWEEN STALK AND SPORE CELL DIFFERENTIATION IN DICTYOSTELIUM DISCOIDEUM, Robert R. Kay, Will Kopachik, Jennifer J. Brookman and Julian D. Gross, Imperial Cancer Research Fund, Burtonhole Lane, London NW7 1AD.

The cells composing a Dictyostelium aggregate differentiate into stalk or spore cells according, it is believed, to the extracellular signals they receive. By inducing the differentiation of totally isolated cells we are identifying these signals. Spore formation in certain mutant strains requires just cyclic AMP, whilst stalk formation requires both cyclic AMP and a hexane-soluble developmentally-regulated factor DIF. We believe that DIF is a "pathway-switching" molecule because partially purified DIF preparations can divert isolated cells from spore to stalk differentiation and because mutants which do not make DIF can embark on spore but not on stalk cell differentiation. Addition of DIF to these mutants allows them to differentiate into normal stalk cells. As in most solid tissues, the differentiated cells in Dictyostelium form a discrete spatial pattern. This pattern could be generated by the restriction of DIF activity to part of the aggregate.

0706 GENE TRANSFER INTO MOUSE TERATOCARCINOMA CELLS, Erwin F. Wagner and Beatrice Mintz, Institute for Cancer Research, Fox Chase, Philadelphia, PA 19111

Teratocarcinoma (TCC) stem cells can function as vehicles for the introduction of specific preselected genes into mice. This has become possible because a recently established euploid TCC cell line, METT-1 (1), has the unique capacity to undergo normal development, including formation of functional germ cells, after the cells are introduced into early embryos (2). In one series of tests, TCC cells selected for thymidine kinase (TK) deficiency were used for DNA-transfer experiments with the plasmid pTKHβG. This vector DNA, which has been successfully introduced into mice via egg-injections (3), contains the human adult β-globin gene and the selectable TK gene of herpes simplex virus. Many transformants were obtained and had stably incorporated several copies of the nonselectable human β-globin gene. Some clones were transcriptionally active and all had retained their capacity to differentiate into various tissues, including hematopoietic cells. In a second set of experiments using wild-type METT-1 cells, dominant-acting vectors, such as pSV2-gpt (4), were applied and were stably integrated into the cells. Thus, any gene of choice can be transferred into TCC stem cells which, after characterization, may be used to produce new mouse strains with predetermined genetic changes.

1) Mintz, B. and C. Cronmiller (1981) Somat. Cell Genetics 7, 489-505.
2) Stewart, T.A. and B. Mintz (1981) Proc. Natl. Acad. Sci. USA 78, 6314-6318.
3) Wagner, E.F., T.A. Stewart and B. Mintz (1981) Proc. Natl. Acad. Sci. USA 78, 5016-5020.
4) Mulligan, R.C. and P. Berg (1980) Science 209, 1422-1427.

0707 FACTORS AFFECTING DNA-MEDIATED TRANSFORMATION OF CHINESE HAMSTER OVARY CELLS. Rodney S. Nairn, Gerald M. Adair, and Ronald M. Humphrey, The Univ. of Texas System Cancer Center, Science Park - Research Division, Smithville, Texas 78957.

We have examined several variables affecting the efficiency of DNA-mediated transformation of Chinese hamster ovary (CHO) cells, including the role of carrier DNA in effecting stable transformation, and the extent of clonal variation in the transformation competence of recipient CHO cell lines. CHO thymidine kinaseless (tk^-) cell lines were transformed with Herpes simplex virus (HSV) thymidine kinase gene in the presence and absence of mouse L cell tk^- carrier DNA, using the calcium phosphate co-precipitation method. In optimal conditions, transformation frequencies of $4x10^{-4}$ were achieved in the presence of carrier DNA. The transformant yield was 1 transformant per ng 3.5 kb HSV tk fragment in these conditions. In the absence of carrier DNA, transformation frequencies were approximately two orders of magnitude lower, and transformant yield was $5x10^{-4}$ transformants per ng HSV tk gene. Southern blotting was used to verify the presence of the HSV tk sequence in transformants, and to examine transformants for the presence of unselected co-transforming sequences. Clonal variation for transformation competence in CHO tk^- recipient cell lines was observed. We have studied this clonal variation for competence in a series of independently-selected diploid and tetraploid CHO tk^- recipient cell lines. This work was supported by NCI grant CA 04484.

0708 SV40-MEDIATED EXPRESSION OF TWO CELLULAR TRANSFORMING GENES Nadia Rosenthal, George Khoury, Ronald Ellis, Thomas Shih, Edward Scolnick, and Peter Gruss. National Cancer Institute, NIH, Bethesda, Maryland 20205.

The transforming gene (V-ras) of the Harvey murine sarcoma virus (HaMuSV) encodes a 21K protein present in both phosphorylated and nonphosphorylated forms (Shih *et al.*, J. Virol. 31: 546, 1971). The V-ras gene bears close homology to two rat cellular sequences; one contains several introns (c-ras 1), while the other is colinear with its transcript (c-ras 2), resembling the v-ras gene. When ligated to the HaMuSV long terminal repeat sequence, the cloned c-ras 1 gene transforms NIH3T3 cells with high efficiency, whereas the c-ras 2 gene transforms very poorly (DeFeo *et al.*, PNAS, in Press, 1981). To investigate the nature of cellular transforming gene expression, SV40 late region was replaced by either of the two cellular genes in both orientations (sense, antisense) with respect to the SV40 late control region. Using an SV40 tsA mutant as helper, viable virus stocks were generated for all four recombinants. Northern blot analysis revealed that both cellular genes inserted in the sense orientation are transcribed in equivalent amounts, but in the antisense orientation, are not transcribed. Protein analysis demonstrated that the sense orientation of c-ras 1 synthesizes an abundant 21K protein. Surprisingly, the sense orientation construct of c-ras 2 produced greatly reduced amounts of a similar 21K protein. This could be explained by a translational defect, (e.g., an additional AUG upstream from the coding sequence, and/or a premature translational stop codon), and may underlie the low transformation efficiency of the c-ras 2 gene.

0709 MOVEMENT OF A BACTERIAL GENE INTO AND OUT OF THE GENOME OF MAMMALIAN CELLS USING AN SV40-PLASMID VECTOR, Martin L. Breitman, Lapchee Tsui, Manuel Buchwald and Louis Siminovitch, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8, Canada.

A recombinant plasmid (pSV2) carrying the SV40 origin of DNA replication and the XGPRT gene of E. coli (Ecogpt) has been stably introduced into HPRT-deficient Chinese hamster cells by DNA-mediated gene transfer. Southern hybridization analyses have indicated that most gpt transformants contain between 1-10 discrete copies of pSV2 associated with high molecular weight (hmw) DNA and all harbor at least one intact copy of the XGPRT gene. Digestion of hmw cellular DNAs with restriction enzymes that cut pSV2 at a single site has suggested that many of the transformants contain tandem copies of pSV2 integrated in a head-to-tail orientation. Although no free plasmid has been detected in these cell lines, replicating low molecular weight DNAs have been isolated following fusion of gpt transformants with simian COS-1 cells. Transformation of E. coli with this DNA has led to the recovery of plasmids indistinguishable from wild type pSV2. Since this DNA also transformed a Gpt^- strain of E. coli to a Gpt^+ phenotype, we conclude that an active bacterial XGPRT gene has been introduced into and rescued from the hmw DNA of Chinese hamster cells.

0710 TRANSFER OF THE OVALUBUMIN GENE INTO MOUSE CELLS BY MICROINJECTION, David D. Brown, Eugene Lai, Bert O'Malley and Edwin C. Murphy, Jr., Dept. Tumor Virology, Univ. Texas M. D. Anderson Hosp. & Tumor Inst., Houston, Texas 77030.
A recombinant plasmid containing the chicken ovalbumin gene and 3 copies of the Herpes thymidine kinase gene (POV12 TK) was introduced into mouse $LMTK^-$ cell nuclei by microinjection. Linearization of the OV plasmid and inclusion in the microinjection mix of a plasmid containing the origin of replication of SV40 greatly increased the frequency of successful gene transfer. However, the frequency of transfer of truncated ovalbumin genes appeared also to be increased by linearization prior to microinjection. Restriction enzyme analysis of the high molecular weight DNA from 4 HAT medium survivor cell clones revealed the presence of one or at best only a few copies of the 12kb ovalbumin gene per mouse genome. Further analysis showed the ovalbumin DNA to be unrearranged. Each of these cell clones produced immunoprecipitable ovalbumin protein and mRNA. However, The amount of ovalbumin produced in these cell clones was not directly correlated with the ovalbumin gene copy number. In fact, clones with only one copy of the ovalbumin gene produced several times the amount of ovalbumin as did the clones with about 5 copies, suggesting that the position of the transferred ovalbumin gene in the mouse genome rather than its frequency dictates the extent to which it functions.

0711 A STRUCTURALLY HETEROGENEOUS FAMILY OF TRANSPOSABLE ELEMENTS WITH TERMINAL INVERTED REPEATS IN SEA URCHINS, Dan Liebermann, Barbara Hoffman-Liebermann, Rob Maxson, Joel Weinthal, Jeff Childs, Stanley Cohen and Laurence Kedes, Howard Hughes Medical Institute and Departments of Medicine and Genetics, Stanford University, Palo Alto, CA. 94305
Recently, a transposable element (TE) was found by us in the middle of an orphon H2B histone gene isolated from a partial lambda phage HAE III library of sea urchin genomic DNA (S. purpuratus). Initial characterization of the TE has shown it to be about 3 kb long and to have inverted repeats at both ends with a minimum length of 110 bp. Nucleotide sequence determination of the TE inverted repeat ends as well as of the TE-H2B histone gene junctions have revealed an internal 15 bp repetitious structure for the inverted repeat ends and a 8 bp sequence duplication of the H2B gene so to give direct repeats flanking the TE, which we refer to as TU1. Initial experiments on the genomic organization of TU1 in the sea urchin genome have shown multiple (200-600) copies whose genomic organization varied among different individuals of the same species. Other experiments in which inverted repeat as well as internal fragments were used as hybridization probes have shown structural heterogeneity of the TEs, have suggested the existence of at least two subgroups of TEs: Those constructed by both middle and inverted repeat sequences and others that contain inverted repeat ends only. Preliminary data, comparing inverted repeat sequences of different TE members, suggest, however, that the inverted repeat ends are highly conserved. Taken together, the above results indicate that the newly discovered family of TEs in the sea urchin differs from most other described eukaryotic TEs which have direct terminal repeats and are homogeneous in structure. The sea urchin TEs resemble, however, the foldback family of TEs in Drosophila.

0712 THE ISOLATION AND CHARACTERIZATION OF CLONES ANOMALOUSLY EXPRESSING THE DIFFERENTIATION ANTIGEN QA 2, Carol L. Reinisch, Lorraine Flaherty and Rene S. Rosenson. The Dept. of Comparative Medicine, Tufts Univ. Sch. Vet. Med., the Dept. of Laboratories and Research, New York State Dept. of Health and the Dept. of Pathology, Harvard Med. School.
Balb/c By($Qa2^-$) mice, when injected with MSV-MuLV-Moloney as weanling animals, develop immunoblastic T cell sarcomas by six months of age(1). Cells isolated from the tumor cell population and cultured at limiting dilution(0.3 cells/well) anomalously express the differentiation antigen Qa2(2). We now report that both clones and lines isolated from the immunoblastic T cell sarcomas express the following cell surface antigens: $I\text{-}A^d$, $I\text{-}E^d$, $H\text{-}2K^d$, $H\text{-}2D^d$. They do not express Thy 1.2, IgG, IgM, IgD or TL. In addition, the cells have receptors for the Fc portion of immunoglobulin and C3b and are esterase positive. Furthermore, gel electrophoresis and autoradiography experiments show that while Ig could not be immunoprecipitated with either IgM or Ig sera, anti MuLV sera precipitated 30K, 65K and 80K dalton proteins typical of MuLV infected cells. These studies show that all the cell lines amd clones isolated from individual Balb/c By mice bearing MSV-MuLV-M induced immmunoblastic T cell sarcomas express the identical phenotype and are in the monocyte-macrophage lineage. This cell type may represent a stage in differentiation which is particularly susceptible to transformation by MSV-MuLV-M or it may be induced and expanded in vivo or in vitro following the onset of retrovirus mediated leukemogenic events. Supported by NIH Grant #21100 and ACS Grant # IM 3000.
1. Reinisch et al. 1980. Amer. J. Path. 101: 265-282.
2. Rosenson et al. 1981. J. Immunol. 126: 2253-2257.

0713 ORIGIN AND HOST RANGE FEATURES OF BIPARTITE SV40 GENOMES, Frank J. O'Neill, Edward B. Maryon, and Dana Carroll, University of Utah and VA Medical Centers, Salt Lake City, Utah 84148.
EL SV40 DNA is contained in two defective but complementing molecules. One contains all of the early (E) and the other all of the late (L) viral sequences. Both contain reiterations of the viral origin and terminus. EL SV40 arises from SV40 defectives generated during diluted virus passage in A172 cells but not during undiluted passage in green monkey TC-7 and BSC-1 cells. Many SV40 defective genomes appear in A172 cells and most contain reiterated origins and termini, but defectives containing either all of the early or all of the late viral sequences do not appear until late passages. The EL SV40 defectives do not overtake other defectives in persistently infected (PI) A172 cells. However, when A172 derived SV40, containing EL genomes, is passaged into green monkey TC-7 cells, PI TC-7 cells emerge. PI TC-7 cells contain EL SV40 as the only defectives and during repeated subcultivations, wt SV40 is apparently lost. Although EL SV40 competes very effectively in TC-7 cells, it grows to low titers, produces small amounts of DNA in Hirt supernates and infection with EL SV40 alone produces only persistent infections. In BSC-1, EL SV40 propagates efficiently with massive cell killing. However, in BSC-1, EL SV40 does not effectively compete with wt and other defective genomes. These results show that EL SV40 has arisen in a cell line where it neither grows efficiently nor competes effectively and suggest that interference activity and persistence of defective SV40 genomes are not conditioned on rapid and efficient multiplication of infectious EL SV40.

0714 THE VIMENTIN GENE IN CHICKEN; Zendra E. Zehner and Bruce M. Paterson, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205
Both cDNA and genomic clones (18 independent isolates) coding for the intermediate filament protein, vimentin, have been isolated in chicken. Coding sequences were confirmed by mRNA selection, isoelectric focusing of selected mRNA translation products, and nucleotide sequencing. Heteroduplex analysis reveals that the various genomic clones contain overlapping DNA sequences. Southern analysis yields only those DNA fragments as predicted by the restriction map of the vimentin gene. Likewise, copy number experiments indicate that the vimentin gene is present in a single copy in the chick genome interspersed with repetitive DNA sequences present at many copies. By a Northern analysis mRNA synthesis increases during muscle development and in RSV-transformed cells. Although the vimentin gene appears to exist as a single copy, two vimentin mRNA species (appooximately 2500 and 2300 nucleotides are detected in all the RNA's tested using either a short cDNA clone (3'-noncoding only) or a longer cDNA clone or a gene fragment as probe. These two mRNA species are equal in abundance even in tissues where the expression of the vimentin gene is low (i.e., pre-fused muscle cultures) or in breast muscle isolated from a single chick. The existence of two vimentin mRNA species is not due to cross-hybridization with desmin. Experiments are in progress to determine how the two vimentin mRNA species arise from the transcription of the single vimentin gene.

0715 ISOLATION OF A PUTATIVE GENOMIC HPRT GENE FRAGMENT BY TRANSFECTION, Douglas J. Jolly, Abby C. Esty and Theodore Friedmann, University of California, San Diego, La Jolla, California 92093.

Human DNA has been used to transfect the human gene for HPRT into mouse HPRT$^-$ cells. DNA from such primary transfectants was used in the same way to generate secondary human HPRT-positive mouse cells. DNA from four such cell lines gave a small number (about 6) bands on Southern blots hybridized to cloned human Alu reiterated sequences or to total human DNA. Several of the bands were common and are presumed to represent portions of the HPRT gene. We have cloned one of these bands, an 11.5 kb R1 fragment in pBR322. Subclones of this fragment without the human repeated sequences have the following properties: (a) they hybridize back to the corresponding 11.5 kb band in Southern blots of DNA from human placenta, all four mouse secondary transfectants, and chinese hamster - human X hybrid cells but not to DNA from LA9 or chinese hamster cells; (b) one subclone hybridized to a single band in blots of RNA from Hela human and two secondary transfectant mouse cell lines, but not to the parent mouse LA9 cell line; (c) in preliminary experiments, selection of messenger RNA from Hela cells and the secondary transfectant mouse cells with this subclone followed by in vitro translation gives a protein band on SDS gels, with apparently the same molecular weight as the human HPRT sub-unit that is not visible in controls. We tentatively conclude that we have isolated an HPRT genomic fragment.

0716 EHANCEMENT OF TK TRANSFORMATION BY SEQUENCES OF BOVINE PAPILLOMA VIRUS DNA. Monika Lusky, Leslie Berg and Mike Botchan, University of California, BerkeleyCA94720

We have chosen to use Bovine Papilloma Virus (BPV) as a model system for studying the effect of autonomous replication on transformation in mammalian cells. BPV DNA is maintained as an autonomous replicon in the plasmid state in multiple copies in transformed rodent cells (Law et al.PNAS 81). Recombinant plasmids carrying the Herpes simplex virus TK gene (HSVTK) with and without the BPV genome were transfected into rodent TK$^-$ cells. The transformation efficiency to TK$^+$ was increased up to 100 fold with BPV-TK plasmids compared to that of TK plasmids alone. In addition the enhancement increased as the stringency of the selective conditions increased. Surprisingly, Southern blot analysis of total cellular DNA of the TK$^+$ cell lines did not show any detectable free plasmid DNA. Thus the enhancement of TK transformation by BPV appears unrelated to autonomous replication. Preliminary experiments using the SV40 system indicate that the BPV genome contains a regulatory element which activates gene expression. The SV40 72 bp repeated sequence upstream from the SV40 early region has been shown to play an essential role for early gene expression. SV40 recombinant plasmids lacking the 72 bp repeat do not express T-antigen at any detectable level and do not replicate in monkey CVI cells.Subcloning of the BPV genome both 5' and 3' to the SV40 A-gene and in either oreintation restored both functions.The sequences responsible for this effect have been mapped to within a 2.9 Kb fragment of BPV DNA. We are attempting to map this regulatory function more precisely. Furthermore we are testing in independent experiments wether the same sequences in the BPV genome are responsible for both effects: enhancement of TK transformation and substitution of the SV40 72 bp region.

0717 SSB and RecA PROTEINS PROVIDE DIRECT INSIGHTS INTO THE CONTROL OF EUKARYOTIC GENES. Jack Griffith, Susan Chrysogelos, and Kathi Dunn. Cancer Research Center, University of North Carolina, Chapel Hill, N.C. 27514.

Structural studies (using electron microscopy and antibodies) of the binding of the E. coli single strand DNA (ssDNA) binding protein to ssDNA reveals that it arranges ssDNA into a repeating chain of particles much like nucleosomes. Each SSB nucleosome contains 145 bases of ssDNA and an octamer of SSB; they are joined by 30 bases of linker DNA. This structure gives rise to micrococcal nuclease and DNaseI patterns very similar to that of eukaryotic chromatin. Another protein, RecA of E. coli also binds ssDNA, but forms a continuous sheath of protein along the ssDNA in a highly cooperative manner. Although RecA binds less tightly to ssDNA than does SSB, the structure which RecA forms allows it to displace SSB. Thus the structure formed rather than the binding affinity of the protein determines which protein can displace the other when both are present. This suggests that chromosomal non-histone proteins of only moderate binding affinity for DNA might bind in the linker regions of chromatin and if they were to form a continuous sheath of protein on the DNA as does RecA, that they might transiently displace the histones, altering the pattern of gene control.

0718 INTERACTION OF RSV WITH THE DEVELOPING CHICK: AN IN OVO REFERENCE FOR TRANSFORMATION STUDIES IN CULTURE, David S. Dolberg and Mina J. Bissell, Lab. Cell Biology, University of California, Berkeley, CA 94720

A system has been developed which enables us to analyze the interaction of viruses with the developing chicken embryo in ovo and to compare this interaction with embryonic cells in culture. RSV (Prague A) was microinjected into 3-4 day old chick embryos (Stage 20) either I.V. or by injection into the limb bud, and the embryos were subsequently prepared as cultures of fibroblasts (CEF) at day 10, tendon cells at day 16, wing web fibroblasts or tendon cells at hatching. Up to 50% of the embryos survive the injection procedure and are capable of proceeding to term. Preliminary experiments have shown: 1) RSV does not appear to be teratogenic or cause tumor growth when injected as described above. Only small hemorrhagic lesions are detected (as described by others); 2) cultures prepared from infected embryos appear normal when plated. However, transformed foci appear within three days post culture. We conclude that: 1) RSV can integrate in at least some cells in the developing chick; 2) unlike cells taken from tumor tissue, cells infected in ovo do not express the transformed phenotype immediately upon culturing; 3) culturing of cells infected in ovo may provide additional factors which allow for the expression of the transformed phenotype. Immuno-histological studies are under way to determine the distribution of viral gene products in ovo. The effects of transformed cells and viral mutants in ovo are also being investigated. Supported by the office of Environment US DOE.

0719 MONOMERIC AND OLIGOMERIC FORMS OF SIMIAN VIRUS 40 T-ANTIGEN HAVE SIMILAR ds-DNA BINDING AFFINITIES, Roland Henning, Mathias Montenarh and Michaela Wachter, Dept. of Biochemistry, University of Ulm, D 7900 Ulm, F.R.G.

Oligomeric and highly aggregated forms of SV40 large T-antigen (16 S-25 S) are more highly phosphorylated than monomeric T-antigen (5-6 S). We observed that highly phosphorylated T-antigen seems to have a higher DNA-binding affinity than less phosphorylated forms. In an attempt to study the influence of different aggregated forms of T-antigen on its DNA-binding affinity we analyzed first the mechanism of the oligomerization. Using chelating reagents we found that oligomeric forms (16-25 S) of T-antigen are formed by subunit interactions via divalent cations whereas a minimal complex between T-antigen and the cellular 56 K protein is stable in absence of divalent cations. Alternatively, oligomeric forms of T-antigen can be dissociated under weakly acidic conditions.

Examination of the binding affinity of T-antigen to ds-calf thymus DNA in crude extracts from SV40-infected and -transformed cells in presence or absence of chelating reagents revealed no distinct differences between either the binding affinities at different pH's or the correlation between the phosphorylation and the binding affinity. These results suggest that oligomeric and monomeric forms of T-antigen seem to have similar ds-DNA-binding affinities. The phosphorylation of T-antigen in the stable complex with the 56 K protein and its DNA-binding properties are under current investigation.

0720 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE HUMAN METALLOTHIONEIN GENE FAMILY, Michael Karin, Metabolic Research Unit, Department of Medicine, UCSF, San Francisco,Ca. 94143.

Metallothioneins (MT) are low molecular weight, cysteine rich, heavy metal binding proteins. To make possible the molecular analysis of the human MT genes, a cDNA "clone bank" was prepared from cadmium induced Hela cells. The cDNA bank was screened using 32P-cDNA probes made from control and induced Hela cells RNA. Colonies that hybridized preferentially with the induced cDNA probe were picked for further analysis by "Southern blotting" of plasmid DNA and hybridization with mouse MT cDNA. One of the positive plasmids was subjected to nucleotide sequence analysis and was found to contain an almost full length cDNA insert of human MT-II. Using the cDNA clone as a probe, a human genomic library was screened, and 53 positive bacteriophage λ clones were isolated. The exact number of absolutely different MT gene clones is not clear yet but seems to be around 10. 2 genes that correspond to the MT-II cDNA were subjected to further analysis. One of them contains 2 intervening sequences and the other is missing them completely. I am currently investigating, using gene transfer techniques whether the two genes are expressable in mouse cells, and whether or not their expression is regulated by heavy metals or glucocorticoids.

0721 INTERACTION OF SV40 PROTEINS WITH VIRAL CHROMATIN, Ann Roman, and Hwa-Tang Wang, Indiana University Medical School, Indianapolis, IN 46223.

We have recently shown that the removal of newly synthesized SV40 chromatin from the replication pathway correlates with its progression along the maturation pathway. We proposed that the fate of newly synthesized chromatin depends upon the relative abundance of initiation and maturation proteins and their affinities for the chromatin template. The effect of mutations in genes coding for early and late viral proteins on the replication and maturation pathways is being investigated. Large T antigen, encoded by the SV40 A gene, is required for initiation of the replication pathway. CV-1 cells infected with a T-antigen mutant (tsA58) were labeled at 33° (permissive) and then shifted to 40° (restrictive). No replication of the labeled chromatin was detected. It was, however, encapsidated with slightly slower or similar kinetics to wild-type SV40.

VP1 is the major structural protein of SV40. Mutations in the gene coding for VP1 prevent the accumulation of intact virions. Analyses of mutations in two different complementation groups, B and BC, have shown that at the restrictive temperature newly synthesized DNA remains in the chromatin form. Removal of DNA from the pool of molecules available for replication, however, occurs with the same kinetics as wild-type SV40. Experiments analyzing the effect of large deletions in late genes are in progress.

The interaction between chromatin and viral proteins may be influenced by the level of acetylation of histones in the chromatin. The effect of sodium butyrate, an inhibitor of deacetylases, on the replication and maturation pathways is being analyzed.

0722 A NOVEL MAMMALIAN DECARIBONUCLEOTIDE POLYMERASE WITH PROPERTIES OF AN iRNA PRIMASE, Gabriel Kaufmann and Hedda Hoffman-Folk, Biochemistry Department, The Weismann Institute, Rehovot, Israel 76100.

Extracts from SV40-infected cells or from calf thymus were found to contain DNA-dependent activity which incorporates UMP residues from UTP into free or DNA-linked decaribonucleotides of random sequence. This incorporation depends on the other rNTPs and was insensitive to both α-amanitin and rifamycin AF/013. Partial purification on ion-exchange resins has separated this activity from RNA polymerases I, II and III. The data implicate the decaribonucleotide polymerase in DNA chain initiation.

0723 REGULATION OF RAT GROWTH HORMONE GENE EXPRESSION STUDIED BY GENE TRANSFER AND SOMATIC CELL HYBRIDIZATION, Peter Kushner, Michael Walker and Howard Goodman, University of California, San Francisco, San Francisco CA 94143

We have introduced an 11 kb Eco Rl fragment of rat genomic DNA containing the growth hormone (GH) gene into mouse tk^- L cells by co-transformation with the herpes simplex virus tk gene. Several independent transformants, including clone L15 which has a single copy of the rat GH gene, synthesize rat GH RNA, while the parental L cells contain no detectable GH RNA. The GH RNA in most transformants increases 2 to 4 fold after addition of the synthetic glucocorticoid dexamethasone, but appears to be about 200 bases shorter than authentic rat GH mRNA prepared from the GH3 pituitary cell line. The transformants make little or no growth hormone protein.

We have also constructed and examined a hybrid cell line, L x GH3-Cl, which retains both the mouse and the rat GH genes. Like other such hybrids, L x GH3-Cl cells make little or no GH protein. Northern blots showed that the hybrid cells entirely lacked GH RNA although GH RNA from the transformant L15 was readily visualized in the same blot. These results indicate that the mouse fibroblast can "extinguish" expression of the rat GH gene at the level of RNA synthesis when the gene is contributed by cell fusion but not when the gene is contributed by co-transformation.

0724 NUCLEOTIDE SEQUENCE ANALYSIS OF INTEGRATED AVIAN MYELOBLASTOSIS VIRUS (AMV) AND AVIAN MYELOCYTOMATOSIS VIRUS (MC29) LONG TERMINAL REPEAT (LTR) AND THEIR HOST AND VIRAL JUNCTIONS: STRUCTURAL SIMILARITIES TO TRANSPOSABLE ELEMENTS. J. A. Lautenberger[1], R. A. Schulz[1], K. P. Samuel[1], D. K. Watson[1], N. C. Kan[1], T. W. Pry[1], M. A. Baluda[2], and T. S. Papas[1]. Laboratory of Molecular Oncology, National Cancer Institute, Bethesda, MD; [2]UCLA School of Medicine, Molecular Biology Institute, Los Angeles, CA

The nucleotide sequence of the integrated avian myeloblastosis virus (AMV) and avian myelocytomatosis virus (MC29) long terminal repeats have been determined. The sequence is 385 bp long for AMV and 315 for MC29. The cell-virus junctions at each end consist of a six base pair direct repeat of cell DNA next to the inverted repeat of viral DNA. The LTR also contains promoter-like sequences, a mRNA capping site, and polyadenylation signals. Several features of this LTR suggest a structural and functional similarity with sequences of transposable and other genetic elements. Comparison of these sequences with LTRs of other avian retroviruses indicates that there is a great variation in the 3' unique sequence (U_3) while the R region and the 5' specific sequences (U_5) are highly conserved. However, the sequence of the MC29 U_3 region is very similar to that of RSV while the AMV U_3 region is not closely related to either evl, RAV2, or RSV.

0725 VARIABLE STRUCTURE OF IVS I IN HUMAN AND APE ζ GLOBIN GENES, Barbara S. Chapman and Allan C. Wilson, Dept. of Biochemistry, University of California, Berkeley, CA 94720

ζ globin genes encode α-like subunits of early embryonic hemoglobin. Although ζ globins and the adult α globins are homologous and their genes form a multigene family on chromosome 16, the structures of ζ and α globin genes are very different. The paired embryonic genes have large intervening sequences and are not the same size. In apes and humans the ζ2 gene is smaller than the ζ1 gene by about 1 kb of DNA, mostly deleted from IVS I. Extensive restriction mapping of genomic DNA from several individuals of various primate species, including humans, reveals a high rate of DNA rearrangement in the ζ globin gene region. DNA insertions and deletions (visible as fragment length polymorphisms) occur at 10-50 times the rate of base substitution in 6-base restriction sites. DNA rearrangements occur primarily in 3 locations, 2 of these are in IVS I of the ζ1 and ζ2 genes and the third is in the 5' flanking DNA of the ζ1 gene. Additional DNA inserted in the 5' flank and IVS I of the human ζ1 gene cloned in plasmid pBRζ does not appear to be repeated elsewhere in the genome. However, a 15 bp sequence of the form ACAG$_G^A$GGXG$_G^A$GGGG, which is associated with length polymorphism in 5' flanking DNA of the human insulin gene, appears as a repeat unit in IVS I of the ζ1 gene. Because the observed DNA fragment length polymorphisms occur in discrete increments of about 0.5 kb, multiplication or deletion of these 15 bp units cannot account for these rearrangements. By sequence analysis of cloned genomic DNA we are attempting to determine whether the repeat units promote unequal recombination of nearby sequences, or serve as recognition sites for DNA transposition.

0726 MITOCHONDRIAL DNA AMPLIFICATION IN THE DEVELOPING BOVINE OOCYTE: A POTENTIAL MECHANISM FOR RAPID GENOTYPIC VARIATION. Paul D. Olivo, Michael V. Van De Walle, Terri L. Armstrong, George S. Michaels, T.K. Lakshmi, Philip J. Laipis and William W. Hauswirth. University of Florida, College of Medicine, Gainesville, FL 32610.

Except for strict maternal inheritance, the segregation of mitochondrial DNA during development and differentiation of the mammalian oocyte is poorly understood. Recently intraspecific variation in mt DNA has been reported, suggesting that a mechanism exists for rapidly altering mitochondrial genotypes. To establish the shortest time span over which such variation is detectable we have compared mt DNA sequences between maternally related Holstein cows. One of two mitochondrial genotypes is found in animals of a single maternal lineage. The genotypes are distinguished by at least 7 single base changes. Six of these occur within the origin of replication, and the remaining change occurs at the third position of a glycine codon. Thus no change as yet characterized leads to altered gene expression. The pattern of occurence of each genotype within the lineage demands that at least 4 shifts between genotypes occurred in the past 20 years. The most rapid shift took place in no more than 2 generations (4 years).

A mechanism for this rapid genotypic shift is suggested by our observation that mt DNA is amplified about 300 fold during bovine oocyte development. We propose that the amplification process may utilize a few, possibly only one, template molecule. Hence, if a female gemate contains low levels of mixed mitochondrial genotypes, a shift is possible in only one generation. Consistent with this model we have detected a low level of mitochondrial heterozygosity in individual animals.

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0727 COMPARISON OF PHENOTYPIC EXPRESSION WITH GENOTYPIC TRANSFORMATION USING CLONED, SELECTABLE MARKERS, Peter S. Linsley and Louis Siminovitch, Department of Genetics, The Hospital for Sick Children, 555 University- Ave., Toronto, Ontario, Canada. M5G 1X8

Two reports in the literature (Pellicer et al (1980) Science 209: 1414-1422 and Chang et al (1982) PNAS, in press) suggested that monitoring phenotypic expression of transfected DNA might be a more sensitive means of detecting DNA sequences than genotypic transformtion. We examined this possibility by comparing phenotypic expression and genotypic transformation with two cloned, selectable markers, HSV-1tk and E. coli gpt. Phenotypic expression was monitored by an autoradiographic assay following transfection of several tk^- and $hprt^-$ cell lines, while genotypic transformation was assessed by scoring HAT^R colonies. With one tk^- line tested (a 3T3 derivitive), and both $hprt^-$ lines (one CHO and one L cell line), the frequency of phenotypic expression was at most 10-fold higher than that of genotypic transformation. The remaining two lines (Ltk^- and 2F3, a CHO tk^- line) showed phenotypic responses 50-100 fold greater than those for genotypic transformation. An evaluation of gene dosage effects indicated that the high frequencies of phenotypic expression reported by others cannot be generalized to these markers. The data provide some insight into processes governing the efficiency of DNA-mediated transformation of mammalian cells.

0728 MOLECULARLY-CLONED GROSS PROVIRAL DNA CONTAINS SEQUENCES HOMOLOGOUS TO XENOTROPIC AKR VIRUS. D.L. Buchhagen and J.D. Morrissey, State University of New York, Downstate Medical Center, Department of Microbiology and Immunology, 450 Clarkson Avenue, Brooklyn, New York 11203.

A stock of Gross MuLV that induces thymic leukemia in 83% of AKR mice within 139 days after inoculation as newborns (119-139 days) and in 42% of C3H/Bi mice was infected into NIH3T3 fibroblasts. An EcoRI-digest of the Gross MuLV-infected cellular DNA was cloned into the arms of λgtWES.λB. One clone, 313, selected by its homology to molecularly-cloned Akv DNA (Lowy et al., PNAS 77: 614-618, 1980), contains an 18 kbp insert. PstI digestion of 313 generates an 8.4 kbp fragment which contains sequences homologous to both ecotropic and xenotropic Akv viruses. Restriction endonuclease mapping of the xenotropic sequences indicate that they are clustered near the 3' end of the PstI fragment. These results are consistent with previous findings of additional RNA genomic information present at the 3' terminus of Gross MuLV but absent from the genome of the nonleukemogenic Akv virus (Buchhagen et al., PNAS 77: 4359-4363, 1980) and suggest that this 3' xenotropic information might play an integral role in the leukemogenicity of Gross MuLV.