MOLECULAR BIOLOGY OF AGING

Organizers: Caleb Finch and Thomas Johnson

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Plenary Sessions Page

Poster Session

Genetics of Lifespan

CG 001 CLONING GENES FOR LIFE-EXTENSION IN *CAENORHABDITIS ELEGANS*, Edward W. Hutchinson, Patricia M. Tedesco, Christopher D. Link and Thomas E. Johnson, Institute for Behavioral Genetics, Box 447, University of Colorado, Boulder, CO 80309

We are exploring the fundamental mechanisms of aging processes through the development and analysis of genetic stocks that have longer life than wild type. These studies are carried out on the simple round worm *Caenorhabditis elegans* which has a normal life span of only 20 days.

Single-gene mutants (1) and genetic lines (2) that have life spans up to 70% longer than wild type have been developed. One gene, *age-1*, has been mapped to a well-studied region of linkage group II. This gene is recessive, affects both males and hermaphrodites and also is responsible for a five-fold decrease in hermaphrodite self-fertility.

This gene is being more precisely mapped by means of multi-factor crosses and deficiency analysis. We are currently cloning the *age-1* gene using a variety of strategies including the construction of congenic strains carrying multiple Tc1-mediated RFLPs flanking *age-1* and deficiencies that uncover *age-1*. We now think that this gene is representative of a larger number of still undetected genes that may function together to limit life. The most significant result of this work is that the limited life of this organism seems to be promoted by the action of the wild-type *age-1* gene product so that elimination of the gene results in prolonged life. The identification of this type of gene corroborates one mechanism implicit in evolutionary models of senescence: that some alleles at some loci can have beneficial actions early in life and shorten life as a pleiotropic effect of that action.

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CG 002 GENETICS AND THE PATHOBIOLOGY OF AGING AND LONGEVITY: AN OVERVIEW, Martin, G.M., Departments of Pathology and Genetics, University of Washington, Seattle, WA 98195. Given the probability that selection for post-reproductive lifespan in iteroparous species was indirect and non-adaptive, the pathophysiologic details of functional declines and of age-related increases in disease susceptibility (and, hence, of length of survival), are likely to vary among species and, to some extent, among individuals within a species. Thus, one can envisage sub-sets of gentic loci, allelic variation and mutation at which might be relevant to the longevity of only a sub-set of related species, to particular species, or to particular individuals within a species (Martin GM, *Exp.Gerontol.* 23:257, 1988). This conclusion does not rule out, however, the possiblity that, among many possible processes of aging, there may exist one or more essentially universal process(es); this is one rationale for investigating the genetic basis of aging in a wide range of organisms. A brief review of such model systems emphasizes the value to gerontology of a more global approach to the genetic analysis of aging and longevity (Martin GM, *J. Gerontol.: Biol. Sci.* 43:B33, 1988). Special emphasis will be given to promising approaches using *Mus musculus* and *Homo sapiens*. Arguments will be developed to support the controversial proposition (eg. see Tully T, *Exp. Gerontol.* 23:B269, 1988) that a genetic analysis of certain age-related diseases may lead to improved understanding of underlying molecular mechanisms of aging. (Supported by NIH Grants AG 00057 , AG 01751 and AG 05136.)

CG 003 GENETICS OF LIFESPAN IN DROSOPHILA MELANOGASTER, Michael R. Rose, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92715 § One of the deepest problems for the study of aging is what it means to study the genetics of aging. Many mutants are known in **Drosophila** which considerably shorten lifespan, particularly mutants of large effect on other aspects of the phenotype, such as body-form. However, it can be argued that these flies are dying because of novel pathological effects associated with mutations of large effect, as opposed to any type of accelerated aging. Indeed, obtaining a true "accelerated aging" mutant would be a profoundly difficult task. § The same is not true of genetic stocks with postponed aging. In these stocks, the processes which normally limit lifespan must be slowed or forestalled in some manner. **D. subobscura** has a mutant allele, grandchildless, which produces females that simultaneously lack ovaries and exhibit longer lifespans. This is somewhat analogous to age-1 in **Caenorhabditis elegans**. Unfortunately, it is generally not feasible to screen for such slower-aging mutants in **Drosophila**, due in part to inbreeding depression arising from linked loci. § An alternative approach is to select for increased lifespan in outbred laboratory cultures by the use of eggs laid only by females that have survived to later ages. The procedure has been by both Rose and Luckinbill to produce stocks with genetically-postponed senescence. The scientific problem then becomes characterizing the genetics of these stocks. Our research has shown that these stocks differ at many loci, these loci having alleles of additive effect, on average. While some research has indicated the existence of a few loci of large effect, those results were statistically artifactual. We have also investigated the physiological mechanisms involved in genetically-enhanced lifespan. The results indicate the involvement of many distinct physiological changes. Some of these changes involve pleiotropically interconnected effects on common mechanisms. But some of these mechanisms also appear to act independently of each other. While the results indicate that there will be no simple global results at the level of physiological mechanisms of aging, they do indicate that it is possible to make considerable progress by means of joint genetic and physiological analysis of selectively-increased lifespan in **Drosophila**.

CG 004 MHC AND AGING IN MAMMALS, Roy L. Walford, Dept. of Pathology, UCLA Medical School, Los Angeles, CA 90024. An early requirement of immunological theory of aging was that the MHC, which regulates much of immunity, would affect maximum life span. Direct evidence thereof, based on survival curves and age-specific incidence of tumors, was obtained in late 70's, using H-2 congenic mice. Regarding mechanisms, the idea that the MHC is primarily an immunoregulatory system reflects its discovery by immunologists. We now know that it influences a variety of non-immune parameters or systems some of which have themselves been implicated in aging: certain free radical scavenging enzymes, mixed function oxidases, DNA repair, and reproductive senescence. To earlier direct evidence based on survival, age-specific tumor and immune biomarker studies may be added the inferential evidence of these additional involvements, that the MHC is one among the as yet undetermined number of gene systems regulating aging in mammals. Recent reports suggest, furthermore, that MHC antigens may act as co-receptors with various hormone receptors, giving the MHC a role in hormonal signal transduction. The MHC has received much attention from molecular immunologists, so that materials for molecular experimentation in gerontology with this system are available. Recent work from my laboratory, or collaboratively with others, on several of the above areas will be presented.

Gene Structure and Aging

CG 005 DNA METHYLATION IN AGING MOUSE LIVER, Laura Mays-Hoopes, Diane Howlett, Stacie Dalrymple, Lisa Spuck, Wei Chao, and Margaret Jennings, Department of Biology, Occidental College, Los Angeles, CA 90041 and James Nelson, Department of OB/Gynecology, McGill, University, Montreal, Canada H3A1A1

DNA methylation has been considered a component of transcriptional regulatory systems, preventing or increasing binding by trans-acting factors(1). Overall DNA methylation, via HPLC, decreases with aging in livers of male C57BL/6J mice (2). Interspersed repeated sequences, examined after methylation-sensitive endonuclease digestion, also show this trend (3,4). Additional sequences (major satellite, minor satellite, and α1 globin) were found to lose methylation with aging, but the timing of demethylation was early for α1 globin and continual for satellite DNA (5). In mouse satellite DNA, methylation-sensitive endonuclease sites, which are rare in these sequences, do not show demethylation, while isolation of the sequences and HPLC does clearly show age-related demethylation. We have not detected increased α1 globin transcripts on dot blots as a function of age, implying that long term demethylation is not sufficient to allow high levels of expression for this gene. Experiments are in progress to determine the limiting factor in age-related demethylation. Liver cells replicate rarely after maturity, so the process of methylation loss is DNA repair followed by incomplete remethylation. We find that the specific activity of liver DNA methyltransferase (DMT) is 4.39 $\pm$ 1.78 in 6-7 month old animals, 4.03 $\pm$ 1.80 in 12.5 month old animals, and 4.21 $\pm$ 1.34 pmol (mg protein)$^{-1}$ (hr)$^{-1}$ in 27-31.5 month old healthy animals. The DMT pH optimum (pH 7.9 - 8.4) and Km for S-adenosyl methionine (23 x 10^{-7} M) were similar, although the thermolability was higher for the oldest animals (young t 1/2 = 8 minutes, 27-31 months t 1/2 = 3 minutes at 42^{0}). The concentrations, determined via HPLC, for the substrate S-adenoysl methionine (22.3 ug/mg liver) and the inhibitor S-adenosyl homocysteine, (51 ug/ml liver) were similar in 3.5 month old and 27 month old mice. We conjecture that locational specifities, regulation of DMT action, or a 5-methyl cytosine glycosylase could explain age-related DNA methylation decreases.

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3. Mays-Hoopes, Chao, Butcher, and Huang (1986). Devel. Genet. 7:65.
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5. Howlett, Dalrymple, and Mays-Hoopes (1988). In press, Mutation Research.

CG 006 ADDUCT-LIKE DNA MODIFICATIONS (I-COMPOUNDS) IN TISSUES OF AGING MAMMALS, Kurt Randerath, Erika Randerath, Donghui Li and Raghu G. Nath, Department of Pharmacology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

I-compounds are recently discovered covalent DNA modifications that are detectable by ^{32}P-postlabeling assay in tissues of untreated experimental animals and tend to increase with age (1-3). Age-dependent increases of these DNA derivatives were initially documented in liver, lung, kidney and heart DNAs of rats (1). To determine which factors may be involved in their formation, I-compounds were examined by ^{32}P-postlabeling in liver and kidney DNA of rats, mice and Syrian hamsters of different ages. The following results were obtained: (i) Every tissue DNA studied contained characteristic I-compounds. (ii) Patterns and amounts of I-compounds differed little among animals of the same kind. (iii) There were pronounced organ and species differences. (iv) I-compound profiles depended on the type of diet (purified versus natural ingredient-). (v) Effects of sex and sex hormones were noted, especially in rat liver and hamster kidney. (vi) As evidenced by their chromatographic properties, I-compounds differed greatly in terms of their polarity, suggesting substantial structural diversity. (vii) Liver I-compound levels were reduced in rats exposed to certain non-mutagenic carcinogens.

These results support the hypothesis that I-compounds are formed by the binding of specific "indigenous" electrophiles to tissue DNA. As persistent DNA alterations, they are likely to affect DNA replication and to play a role in spontaneous and chemically-induced carcinogenesis and in aging.

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DNA Repair and Aging

CG 007 DNA REPAIR IN RELATION TO GENE EXPRESSION IN DIFFERENTIATED CELLS, Philip C. Hanawalt, Pauline Gee, and Linus Ho, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

Overall genomic DNA repair activity may not be a good indicator of cellular sensitivity to DNA damaging agents and is unlikely to be of causal significance in the process of aging. However, there may be subtle changes in the pattern of preferential repair of genes with aging, especially when the maturation process is coupled with cessation of replication, and those changes could account for some age-related changes in cellular responses to damage. Changes in the classes of genes repaired during differentiation may have an indirect effect on aging as a programmed maturation process. Therefore, it is of interest to understand how the fine structure of DNA repair is affected by terminal differentiation. We have studied this question in several model systems: L8 rat myoblasts differentiated to myotubes by serum deprivation and PC12 (rat pheochromocytoma) cells differentiated by nerve growth factor (NGF). One of the genes induced to high levels of expression by NGF in PC12 cells codes for a 43k dalton growth associated protein (GAP-43) which migrates with the growth cone as neurites form neural networks. Using a cDNA clone of this gene as a probe for quantitative hybridization analyses of Southern blots, we found that 50% of the pyrimidine dimers were removed in 72 h after exposure to 20 Jm^{-2} of UV irradiation. In untreated cells, in which the GAP-43 gene is expressed only at basal levels we found 30% repair in the same repair interval. Expression of synapsin I, another gene induced by NGF, correlates with neuronal maturation. Using similiar hybridization analyses, we found 55% repair in 48 h. In L8 cells, DNA repair in differentiating myotubes appears to be efficient for all specific gene sequences so far examined after sufficiently long repair periods (up to five days), regardless of transcriptional activity. However, the kinetics of repair differ between transcribed and non-transcribed sequences, the repair of the induced embryonic myosin heavy chain (MHC_{emb}) gene being more rapid than that of the non-transcribed serum albumin gene or the GAP43 gene, for example. Further analyses in these differentiating cell systems should contribute to our understanding of not only how cells cope with genotoxicity but may also provide clues as to which genes remain important to cells which are no longer actively proliferating in the aging organism. (Supported by NIH grants, CA 44349 and AG 05568)

CG 008 DNA REPAIR IN THE NEMATODE CAENORHABDITIS ELEGANS, Phil Hartman, Department of Biology, Texas Christian University, Fort Worth, TX 76129

The small free-living nematode C. elegans is a popular model for the study of many biological phenomena, including DNA repair. Relatively little is known about DNA repair in C. elegans when compared with mammalian cells; however, as will be described, such studies should reveal additional complexities about DNA repair, particularly developmentally related aspects.

Three major DNA repair systems exist in most organisms (photoreactivation, excision repair and post-replication repair). Their functional status in C. elegans has been determined. Three independent approaches indicated that this metazoan lacks light repair (photoreactivation). Excision repair was assayed using a radioimmunoassay that quantitates both cyclobutane dimers and (6-4)photoproducts, the two major UV radiation-induced DNA damages. Wild-type embryos excised approximately 90% of both photoproducts by 24 hours after irradiation; repair capacity declined slightly throughout development. Of four radiation-sensitive (rad) mutants tested, only rad-3 was excision-repair defective. Post-replication repair was determined by pulse-labeling irradiated embryos and measuring the molecular weights of newly synthesized DNA's using alkaline sucrose gradient centrifugation. The DNA's from irradiated and unirradiated embryos were chased into high molecular weight DNA at approximately equal rates, indicating that C. elegans possesses an efficient post-replication DNA repair system. Curiously, doses of up to 270 Jm^{-2} failed to reduce the molecular weight of newly synthesized DNA relative to that of unirradiated controls. Since numerous lesions were induced in the template opposite each nascent fragment, C. elegans may be in unique possession of a DNA polymerase capable of translesion synthesis. This would explain the observation that embryonic DNA synthesis in this nematode is over ten times more resistant to UV irradiation as compared with other model systems (e.g., yeast, mammalian cells, bacteria).

The relationship between DNA repair and aging has been examined in several ways. First, the lifespans of the rad mutants were determined and, for several mutants, were found to be normal. Second, five strains with mean life spans ranging from 13 to 30.9 days were found to have experimentally identical sensitivities to several DNA-damaging agents. Finally, the radiation sensitivities of a long-lived developmental stage (dauer larvae) did not change over the course of months. These data indicate that DNA repair plays a minimal role in the normal process of aging in C. elegans.

Gene Regulation and Aging-I

CG 009 EXPRESSION OF HUMAN PLASMA PROTEIN GENES IN TRANSGENIC MICE

Barbara H. Bowman, Gwendolyn S. Adrian, Robert M. Riehl*, LeAnn Robinson, Christi A. Walter, Damon C. Herbert, Frank J. Weaker, Erle K. Adrian, Carl A. Eddy*, Carl J. Pauerstein* and Funmei Yang, Department of Cellular and Structural Biology and *Department of Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, Texas 78284

The human plasma protein genes offer excellent models for studying gene expression during ontogeny, acute-phase reaction, hormonal modulation and aging. A major plasma protein, transferrin (TF), is required by vertebrates to carry iron from intestine, reticuloendothelial system and liver parenchymal cells to all proliferating and iron-requiring cells in the body. The aim of the work described here was to utilize transgenic mice to determine those regions of *TF* DNA that respond *in vivo* to cellular signals affecting plasma protein synthesis in specific tissues during the aging process.

Two transgenes were introduced into the germline of mice. Gene expression was analyzed in transgenic mice carrying chimeric genes composed of two different lengths, 0.67 and 1.2 kb of the 5' flanking region, of the human transferrin (*TF*) gene joined to the reporter *CAT* (chloramphenicol acetyl transferase) gene. It was observed that in transgenic mice the DNA sequence within 0.67 kb of the 5' flanking region of the human *TF* gene is adequate to activate *CAT* gene expression in appropriate tissues. Expression of *TF(0.67kb)* and *(1.2kb)CAT* chimeric genes in eight lines of transgenic mice demonstrated tissue specificity, consistent with synthesis of endogenous transferrin in mouse liver, brain and testis. Both endogenous transferrin synthesis and *TF-CAT* gene expression were also discovered in heart and thymus of transgenic mice.

The highest expression of the mouse endogenous gene occurs in liver, whereas expression of both *TF(0.67kb)CAT* and *TF(1.2kb)CAT* chimeric genes was greatest in brain, especially in the cerebellum. The TF(1.2) sequence, especially, appears to be a robust promoter that may prove useful for expressing structural genes in brain tissue.

In mouse embryos endogenous transferrin synthesis and *TF(1.2 kb) CAT* gene expression have been observed for the first time at the 8 cell stage to the expanded blastocyst stage of embryogenesis. Transgenic mouse lines carrying the *TF-CAT* genes, therefore, will provide helpful models for studying transferrin expression in different cell types during embryogenesis, maturity and into the aging process.

CG 010 THE EFFECT OF AGING AND DIETARY RESTRICTION OF GENE EXPRESSION BY LIVER TISSUE FROM MALE RATS, Arlan Richardson, Govinda Rao, Ahmad Heydari, Erning Xia, and Bo Wu, Department of Chemistry, Illinois State University, Normal, IL 61761.

Dietary restriction (underfeeding not malnutrition) is the only experimental manipulation known to increase the longevity of mammals. It has been shown to have a profound effect on a variety of physiological and pathological processes in all tissues. Thus, the increase in longevity appears to arise from an alteration in the rate that an organism ages. Although it is well documented that dietary restriction increases the longevity of laboratory rodents, the molecular mechanism underlying the action of dietary restriction is not known.

We proposed that changes in gene expression might be an important factor in the mechanism underlying dietary restriction because gene expression is a common site of regulation in all cells/tissues and because changes in gene expression can markedly affect cellular functions. In addition, gene expression is altered in many tissues as an organism ages.

The effect of dietary restriction on age-related changes in the expression of a variety of genes has been studied in liver tissue isolated from male Fischer F344 rats. The rats were fed either ad libitum or 60% of the diet consumed by the rats fed ad libitum (restricted diet). This dietary restriction regimen increased the longevity of the rats over 30%. Dietary restriction retarded the age-related decline in the expression of α_{2u}-globulin. The synthesis, mRNA levels, transcription of α_{2u}-globulin genes was enhanced 2-fold by dietary restriction. Dietary restriction also increased the expression of superoxide dismutase and catlase. The enzyme activities, mRNA levels, and nuclear transcription of these two genes was increased approximately 50% by dietary restriction. However, the age-related increase in the expression (mRNA levels) of apolipoprotein B and the oncogene, c-myc, were not altered by dietary restriction. Recently, we found that the induction of the heat shock protein HSP 70 by hepatocytes declined markedly with age. Currently, we are studying the effect of dietary restriction of the induction of this protein, which could have important physiological consequences to the organism.

Our research clearly shows that dietary restriction can alter gene expression at the level of transcription. There is no evidence that changes in gene expression are responsible for the increased survival observed for rats fed the restricted diet; however, one would predict that the increased expression of superoxide dismutase and catalase would be beneficial to the organism. (Supported in part by NIH grant AG 01548)

CG 011 **TISSUE SPECIFICITIES AND DEVELOPMENTAL PATTERNS OF HUMAN β-AMYLOID GENE EXPRESSION.** Axel J.Unterbeck, Dana O.Wirak, Richard M.Bayney, Bruce D.Trapp*, Edward H.Koo°, Donald L.Price°, and George Scangos, Molecular Therapeutics Inc., West Haven, CT 06516,*Department of Neurology,The Johns Hopkins University School of Medicine,Baltimore,MD 21205, °Departments of Pathology, Neurology and Neuroscience,The Johns Hopkins University School of Medicine, Baltimore,MD 21205-2182

We are investigating the relationship between the amyloid precursor protein (APP) and the formation of amyloid in Alzheimer's disease. Experiments will be presented to study the developmental regulation and tissue-specific expression of the APP gene in vivo, and to assess the relative levels of alternative transcripts derived from the APP gene in normal and pathological brain tissue.

Various gene constructs containing the APP regulatory region and APP gene transcripts have been introduced into mice. These transgenic mice provide a unique opportunity for studying the differential expression pattern of the APP gene, and for studying the biological effects of this gene in vivo. Furthermore, these animals can be used to generate models of disease, which will be discussed.

Gene Regulation and Aging-II

CG 012 MOLECULAR BASIS OF IMMUNESENESCENCE, Marc E. Weksler, Rise Schwab, David Gamble and Paul Szabo, Department of Medicine, Cornell University Medical College, New York,N.Y. 10021.

The molecular basis of the impaired proliferation of T lymphocytes from humans over 65 years of age will be discussed. Specifically, we shall report age-associated differences in two early steps of lymphocyte activation: (I) the transcription of the c-myc proto-oncogene and (II) the expression of the high affinity receptor for IL-2.

In the presence of PHA the steady state level of c-myc message measured by dot blot filter hybridization using a probe from the second exon of the c-myc genomic locus was found to be 50% lower in T-lymphocytes from old as compared to young donors. In situ hybridization using this probe showed that approximately half as many lymphocytes from elderly donors had increased c-myc levels. Data from nuclear transcription analysis showed that the difference in the steady state of c-myc RNA correlated with the rate of transcription of the second exon suggesting that the steady state levels of c-myc mRNA is regulated at the transcriptional level. In contrast, the rate of transcription of the first exon was 4 to 8 times greater than downstream sequences suggesting a defect in elongation of initiated transcripts.

The interaction of IL-2 with its receptor is critical for the proliferation of T lymphocytes. Activated human T lymphocytes express the 55 kd and 75 kd proteins that make up the high affinity IL-2R on the same number of T lymphocytes and at the same density on lymphocytes from young and old donors. However, there are approximately 50% fewer T lymphocytes that express the high affinity IL-2R. The defect in the expression of high affinity IL-2R appears be due to the impaired association of the two molecules that together make up the high affinity IL-2R.

These results suggest that there are approximately one-half as many T lymphocytes from old as compared to young that are activated in culture with PHA. Two early stages in T cell activation, associated with the passage of these cells through the G1 phase of the cell cycle, are impaired in approximately half the T lymphocytes from old donors. The in vitro proliferative defect in T lymphocytes from elderly donors precedes the transcription of the second exon of the c-myc proto-oncogene.

Viruses and Aging

CG 013 PRION DISEASES AND AGING, Stanley B. Prusiner, Department of Neurology, University of California, San Francisco, CA 94143-0518

The neurodegenerative diseases kuru, Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler syndrome (GSS) are all caused by prions. In CJD, neurologic dysfunction is first manifest between the ages of 50 and 65 while in GSS this generally occurs between the ages of 40 and 60. GSS and familial CJD are autosomal dominant disorders and they are the only known human diseases which are both inherited and transmissible. The most well studied prion disease is scrapie of sheep and goats. The scrapie prion protein (PrP^{Sc}) is the only component of the infectious scrapie prion identified, to date. Although much biochemical and genetic evidence argues that PrP^{Sc} is a major component of the infectious particle, the most convincing data is derived from immunoaffinity purification studies (1). After dispersion of brain microsomes isolated from scrapie-infected hamsters into detergent-lipid-protein complexes (DLPC), copurification of PrP^{Sc} and scrapie infectivity was obtained with PrP 27-30 monoclonal antibody affinity columns. Polyclonal rabbit PrP antiserum reduced scrapie infectivity dispersed into DLPC by a factor of 100. While these results and those of other studies establish that PrP^{Sc} is a component of the infectious particle, the possibility of a second component such as a small nucleic acid which might be required for infection must still be considered. PrP^{Sc} is encoded by a single copy cellular gene and not by a hypothetical nucleic acid within purified prion preparations (2). Normal, uninfected cells express the cellular prion protein (PrP^{C}). Both PrP^{Sc} and PrP^{C} appear to be translated from the same 2.1-kb mRNA. The *N*-terminal amino acid sequences of hamster PrP^{C} and PrP^{Sc} are identical (3); both correspond to that predicted by the translated prion protein (PrP) gene sequence (4). While the chemical difference between PrP^{C} and PrP^{Sc} remains unknown, the organization of the PrP gene argues that it results from a posttranslational event. The mouse PrP gene is on chromosome 2 and is linked to a gene controlling the scrapie incubation time (*Prn-i*) (5). PrP genes from inbred mice with short and long incubation times differ at codons 108 and 189, a finding consistent with but not proving that PrP modulates susceptibility to scrapie (6). PrP^{Sc} stimulation of a posttranslational process which converts PrP^{C} or its precursor into PrP^{Sc} is one possible mechanism for prion replication. This is consistent with observations showing that human prion diseases are manifest as infectious, sporadic and genetic disorders.

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Molecular and Cell (Clonal) Lifespans

CG 014 AGING OF THE DIFFERENTIATING FIBROBLAST STEM CELLS IN VIVO AND IN VITRO, Klaus Bayreuther, Institut für Genetik, Universität Hohenheim, D7000 Stuttgart 70, F.R.G.

The fibroblast stem cell systems in skin and lung of Valo chicken, C3H mice, BN-rats and man in vivo and in vitro have been found to be composed of nine cells in four compartments. In these four compartments the cells differentiate along a nine stage differentiation sequence. Stemcells S1 - S2 in the stem cell compartment, mitotic fibroblasts (MF) MF I - MF II - MF III in the proliferating and differentiating compartment, postmitotic fibroblasts (PMF) PMF IV - PMF V - PMF VI in the postmitotic and maturing compartment, and postmitotic and degenerating fibroblasts (PMF) PMF VII in the degenerating compartment. The morphologically recognisable cell types MF I-PMF VII are different in morphological and biological properties and in 11 biochemical parameters, e.g. in 2-d-gel electrophoresis pattern of nuclear-, cytoplasmic-, membrane-, and secreted proteins or by monoclonal antibodies for fibroblast celltype specific antigens. In normal dermis in vivo the mitotic fibroblasts MF I - MF II - MF III are evenly distributed in the reticular and papillary layer, the postmitotic fibroblasts PMF IV - PMF V - PMF VI - PMF VII are restricted to the boundary zone between dermis and epidermis. As a function of the in vivo and in vitro age a very significant decrease of the cells of the three proliferating compartments occurs.

CG 015 NEGATIVE GROWTH CONTROL IN CELLULAR SENESCENCE, J.R. Smith and O.M. Pereira-Smith, Department of Virology and Epidemiology, Baylor College of Medicine, One Baylor Plaza, Houston,TX 77030.
Fusion of normal human fibroblasts with any number of immortal human cell lines yielded hybrids that had limited division potential in culture. This result indicated that the phenotype of cellular senescence was dominant, and that cellular immortality resulted from recessive changes in the cell. This idea was further tested by fusing different immortal cell lines with each other, and led to the identification of at least 4 complementation groups for cellular immortality. When cell lines within a complementation group were fused with each other, immortal hybrids were obtained and when fusions were performed between groups, hybrids with finite lifespan were obtained. Therefore, there are at least 4 pathways to cellular immortality. In a separate series of experiments, senescent cells have been found to produce a protein inhibitor(s) of initiation of DNA synthesis, and to contain a high abundance of mRNA(s) that inhibits DNA synthesis. The mRNA(s) appears to act by inhibiting the initiation of DNA synthesis, as assayed by micro-injection into young HDF cells. We are currently undertaking the isolation of the senescent cell inhibitor protein and cDNAs of the gene that codes for it.

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Cell Death

CG 016 **Cellular Mechanisms of Hypoxic and Anoxic Cell Injury.** **Brian Herman and John J. Lemasters, Department of Cell Biology & Anatomy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7090.**

The cellular mechanisms governing anoxic and hypoxic injury in cells are not well understood. Events which might contribute to injury include alterations in cytosolic free calcium, cell swelling, oxidative stress, phospholipid degradation, proteolysis and mitochondrial damage. Using the technique of Multiparamenter Digitized Video Microscopy (MDVM) and fluorescence assays of cell viability, we have monitored directly changes in cytosolic free calcium, intracellular pH, mitochondrial membrane potential, cell swelling, changes in cell morphology and cell killing which attend true anoxia or chemical hypoxia (KCN and iodoacetate) in single intact hepatocytes. We have also examined a large number of pharmacologic agents for protection against the onset of cell death due to hypoxic and anoxic injury. Our results indicate: 1) a rise in cytosolic free calcium is not part of the pathway leading to cell death due to hypoxia, 2) cell killing is a rapid event, synonomous with the onset of irreversible injury, and is precipitated by a breakdown of the plasma membrane permeability barrier, 3) intracellular pH drops by 0.5-1.0 pH units very quickly in anoxia and protects against cell death, 4) cell swelling is not an integral step leading to irreversible injury, and 5) proteolysis, phospholipid degradation and reductive stress promoting oxygen radical formation may be critical events leading to lethal hypoxic and ischemic injury.

Molecular Approaches to Age-Related Diseases

CG 017 "MOLECULAR PATHOLOGY" OF AMYLOID A4 PROTEIN IN AGING, ALZHEIMER'S DISEASE AND DOWN'S SYNDROME, Konrad Beyreuther[1] and Colin L. Masters[2], [1]Center for Molecular Biology, University of Heidelberg, INF 282, D-69 Heidelberg, F.R.Germany; [2]Department of Pathology, University of Western Australia, Perth, Western Australia 6009

Alzheimer's disease (AD) is characterized by massive deposition of the 42 to 43 residue amyloid A4 protein (also known as β protein) in the brain. The A4 protein is derived by proteolytic cleavage from the extracellular and transmembrane domains of much larger precursors (Pre A4) which are encoded by the PAD (APP) gene on the long arm of chromosome 21. A similar process occurs at an early age in all individuals with Down's syndrome (DS). There are two distinct pathways involved in the pathogenesis of AD. The intracellular processing of PreA4 gives rise to A4 aggregates which form neurofibrillary tangles. The extracellular processing of PreA4 gives rise to either amyloid plaques or vascular amyloid. It is most likely that the main origin of the extracellular material is also neuronal since PreA4 is detected in brain sections in neurons and at synapses. Since neurons and synapses are the clinical relevant target sites for AD, amyloid A4 deposition in neurons and between synapses would be a crucial event for the expression of dementia in AD and DS. Damage to membranes and factors interfering with the normal biosynthesis and catabolism of PreA4 as the putative primary lesion required to start the amyloidogenic pathway together with the slow evolution of amyloid deposits may account for the age-relatedness of a process that leads to AD.

CG 018 ADVANCED NONENZYMATIC GLYCOSYLATION OF PROTEINS: ROLE IN LATE COMPLICATIONS OF AGING AND DIABETES, Helen Vlassara, Laboratory of Medical Biochemistry, Rockefeller University, New York, NY 10021

Recent evidence on the chemistry and biology of nonenzymatic glycosylation of proteins suggests that this process may be largely responsible for the major irreversible pathologic features of long-term vascular complications associated with diabetes and aging, through formation of irreversible Advanced Glycosylation Endproducts (AGE). Such adducts continue to accumulate as a function of time and glucose concentration on long-lived proteins, such as collagen, basement membrane proteins, lens crystallin, and myelin proteins, leading to a number of processes associated with aging, i.e. thickening of glomerular and capillary basement membrane, arterio- and atherosclerosis, periarticular rigidity, and peripheral neuropathy. In addition, these modifications may contribute to age-related pathology by altering the structure and function of genetic material. Recently, the pharmacologic agent aminoguanidine HCl was found to effectively inhibit the formation of AGE.

Nonenzymatically glycosylated proteins have been shown to be specifically bound and internalized by mouse and human macrophages through a recently characterized and isolated high affinity receptor (m.w. 90kD). Insulin appears to be a potent suppressor of this macrophage AGE-receptor activity. This may explain in part the association between high insulin levels and vascular complications noted in individuals with insulin resistance and mild glucose intolerance.

In addition, we have recently demonstrated that coupling of AGE-proteins to its receptor results in TNF and IL-1 synthesis and secretion. This suggests that glucose-induced modifications forming on long-lived proteins may be the signals that stimulate growth-promoting factors during tissue remodelling. In aging and diabetes, an imbalance between proteolytic and synthetic processes could lead to the excessive proliferative changes seen in the vascular wall. Furthermore, a marked increase in maximum binding and degradation of nonenzymatically glycosylated albumin (AGE-BSA) by macrophage/monocytes previously exposed to TNF was demonstrated as compared to non-exposed cells. These effects were completely blocked in the presence of an anti-TNF antibody. These data suggest that AGE-induced TNF may normally play an important regulatory role on the macrophage removal of glucose-mediated modifications forming on senescent tissue proteins, such as in vessel walls.

The effect of aging on the macrophage AGE-receptor was evaluated in young (6-months old) and old (2.5-years old) mice. A greater than two-fold decrease in both receptor number and binding affinity was found in cells from the old group as compared to the young group of animals. These data suggest that aging in itself may adversely affect the AGE-receptor efficiency, which may compound age-related tissue damage by preventing the removal of crosslinked glycosylated proteins.

Genetics and Allelic Variants in Aging Processes

CG 100 AGE-ASSOCIATED CHANGES IN DNA POLYMERASE ALPHA ISOZYME EXPRESSION, David Busbee, Geoffrey Curtin, James Norman, Vinod Srivastava, and Roger Tilley, Division of Cell Biology, Department of Anatomy, Texas Veterinary Medical Center, Texas A & M University, College Station, TX 78743. Eukaryotic DNA polymerase alpha is a major enzyme which adds nucleotides to an open 3'-OH end during DNA synthesis. Available data suggest that DNA polymerase delta may catalyze synthesis of the leading strand, while DNA polymerase alpha catalyzes synthesis of the lagging strand and acts to resynthesize the majority of the excised segment of oligonucleotide during "long patch" DNA excision repair. DNA polymerase alpha has been reported by this and other laboratories to exist in multiple forms which may be dependent on the stage of the cell cycle at which analysis occurs, and is dependent on both the cell donor age and the in vitro age (passage number) of the cells. DNA polymerase alpha isozyme forms which have been reported to be highly active (fetal; A2) or less active (adult; A1) differ in their relative intracellular ratios dependent on the donor age of the cells. DNA polymerase alpha A1, but not A2, can be activated by treatment with phosphatidylinositol-4-monophosphate (PIP) or its hydrolysis product inositol-1,4-bisphosphate (IP2), with a concomitant increase in fidelity of the enzyme.

CG 101 ALLELIC VARIATION IN PEROXIDASE OF DROSOPHILA MELANOGASTER DURING DEVELOPMENT AND AGING, Linda K. Dixon, Department of Biology, University of Colorado at Denver, Denver, CO 80204 Variant forms of peroxidase in Drosophila have been identified by two methods: spectrophotometry and electrophoresis. By monitoring photometrically the reaction of H_2O_2 with p-phenylenediamine, we found three major variants of peroxidase with pH optima of 5.9, 7.4, and 8.9. A fourth small peak was seen at pH 5.0. Each of the three major variants had distinct biochemical properties; in addition, pairwise comparisons of activities of the three across 13 Drosophila strains showed no significant correlations, suggesting the three variants are translationally distinct isozymes. Only neutral peroxidase (pH 7.4) showed significant changes with age. Further studies of peroxidase by electrophoresis on agarose gels, using diaminobenzidine as substrate, yielded four distinct bands which varied during preeclosure development. In nine strains of flies, developmental changes were seen in third instar larvae, early pupae, and middle pupae. Each of the different stages had different patterns of bands predominating. In one strain two of the isozymes were absent in the three stages studied. After eclosure, the isozymes became membrane bound, as shown by cross immunoelectrophoresis. Thus, the electrophoretic isozymes have not been monitored during aging. We are presently trying to determine whether the two methods are yielding the same variant forms of peroxidase in Drosophila.

CG 102 AGE-RELATED AND TISSUE-SPECIFIC VARIATIONS IN REPETITIVE SEQUENCES OF MOUSE EXTRACHROMOSOMAL CIRCULAR DNAs, James W. Gaubatz and Sonia C. Flores, Department of Biochemistry, University of South Alabama, Mobile, Alabama 36688. Extrachromosomal circular (ecc) DNAs appear to be a ubiquitous feature of higher cells. Previous studies using cultured animal cells have shown that circle size and abundance can vary as a function of growth conditions or metabolic status. We have initiated an analysis of eccDNAs in vivo to assess the utility of these molecules as biomarkers for mammalian aging. eccDNAs were isolated and characterized from 1-mo., 8-mo., 16-mo., and 24-mo. C57BL/6 mouse heart, liver and brain tissues. DNA preparations were probed for repetitive sequence families complementary to short-interspersed (B1 and B2), long-interspersed (L1), endogenous retroviral (IAP), and tandemly-repetitive satellite (SAT) sequences. Together these sequence families comprise 20% of the mouse genome. Hybridization results showed that each tissue had a characteristic representation of repetitive sequence elements in eccDNAs. Repetitive sequences decreased significantly in liver and brain eccDNAs from 1-mo. to 8-mo. of age but remained stable thereafter. In contrast, repetitive sequence families in heart eccDNAs were constant from 1-mo. to 16-mo. of age but declined 50% in 24-mo. old mice. Electron microscopic analyses demonstrated that heart eccDNAs had similar size distributions at all ages; however more discrete, larger size classes were observed in 24-mo. preparations, suggesting that eccDNAs were altered in senescent myocytes. The data indicate that repetitive sequences in eccDNAs are regulated in a cell-specific manner, and the properties of such molecules may indeed reflect a cell's differentiated state. (Supported by a grant from AFAR, Inc.).

CG 103 **QUANTITATIVE GENETICS OF AGING IN DROSOPHILA**, E.W. Hutchinson, Institute for Behavioral Genetics, University of Colorado at Boulder, Boulder, CO 80309-0447.
The quantitative genetics of postponed senescence in Drosophila melanogaster were investigated. Trade-offs between genetic effects on early fitness and later senescence had been found in past experiments, suggesting pleiotropic interrelations between early and late life history characters. The genetic basis of such life history responses to laboratory selection were studied by means of population crosses using postponed-senescence stocks and matched controls. There was little evidence of non-Mendelian inheritance, inbreeding depression, net directional dominance, or sex-linkage. The apparently simple additive inheritance of postponed senescence allowed the use of conventional quantitative genetic estimators for gene number. estimators are Assays were performed on 24-hour fecundity, ovary weight, female starvation resistance, male starvation resistance, female longevity, and male longevity. The results indicate more than one loci involved in postponed aging. Heritability estimates revealed abundant genetic variability in both selected and control lines, indicating that neither were near fixation. Selection experiments designed to push each of the two sets of lines towards fixation were performed. A genetic analysis performed on the newly selected lines again showed little evidence of non-Mendelian inheritance, inbreeding depression, net directional dominance, or sex-linkage linkage. The results for this second set of gene number experiments also indicated that there was more than one locus, or genetic unit, involved in the postponed senescence response. Therefore, the system appears to be polygenic, with the alleles having additive effects.

CG 104 REORGANIZATION IN THE DIFFERENT HIERARCHICAL STRUCTURES OF DNA DURING CELL SENESCENCE. A. Macieira-Coelho, Department of Cell Biology Faculty of Health Sciences, 581 85 Linköping, Sweden.
Our results on the effect of ionizing radiation and on the kinetics of cell division during aging of proliferative cell compartments, suggested that the cell life span depends primarily upon the genome reorganization occurring during the cell cycle. Indeed the experiments we deed along these lines support our working hypothesis. We found that the distribution of DNA between daughter cells is asymmetric in a significant fraction of cells at each population doubling and that deviations from semi-conservative synthesis are a source of cell heterogeneity. A reorganization could be ascertained at the level of the 30 nm chromatin fibers. Using an image processor to screen electron microscopic pictures of the solenoïde structure, we established a correlation between the reorganization of chromatin and the changes in the initiation of DNA synthesis. The 10 nm chromatin fibers were also analysed and the results were compatible with changes in chromatin conformation occurring through cell divisions. Two genes that are expressed (beta-actin and beta-interferon) and one that is not expressed (alpha-globin) were followed with hybridization with cDNA probes. An attenuation of the hybridization signals was found with the beta-actin and alpha-globin genes. No alteration could be observed with the beta-interferon gene.

CG 105 **A GENETIC AND MOLECULAR ANALYSIS OF ACATALASEMIA IN *DROSOPHILA MELANOGASTER*: TESTING THE OXYGEN FREE RADICAL THEORY OF AGING**, **William J. Mackay and Glenn C. Bewley, Department of Genetics, North Carolina State University 27695-7614.** **Activated oxygen species have been demonstrated to be the important agents in oxygen toxicity by disrupting the structural and functional integrity of aerobic cells through lipid peroxidation events, DNA damage and protein inactivation. The accumulated affect of oxygen free radical damage is thought to be a contributing factor to aging, carcinogenesis and tumor promotion, and an ever increasing list of aging-related disorders. We are developing genetic models for antioxidant enzymes that will provide an important source of material to directly access the role of free radical damage in biological aging and if antioxidant enzymes play a significant role in minimizing these effects. Catalase (H_2O_2:H_2O_2 oxidoreductase, E.C. 1.11.1.6) is one of several enzymes involved in scavenging activated oxygen species. We have isolated six independently-derived acatalasemic mutants in *Drosophila melanogaster*. These mutants are viable under standard laboratory conditions but exhibit extremely shortened life spans. We are currently interested in the role of catalase in protecting Drosophila from DNA damage and the relationship between oxyradical induced DNA damage and lifespan determination. This relationship will be examined by measuring both the germ-line and somatic mutation rate in acatalasemic mutants relative to wild type. We have cloned and are currently conducting a molecular analysis of the catalase gene. To test the prediction that overexpression of antioxidant enzymes can lengthen the maximum life span potential of Drosophila, multiple copies of the catalase gene will be introduced into the Drosophila genome by P-element mediated transformation to determine the effect of overexpression on Drosophila lifespan and mutation rate.**

CG 106 MUTATIONAL ANALYSIS OF HUMAN COLONY STIMULATING FACTOR 1 RECEPTOR

Alan Y. Sakaguchi, Ling-Mei Wang, Dawn K. Gheil, Ann Minter and Goutam Ghosh-Choudhury, Department of Cellular and Structural Biology, The Univ. Texas Health Science Center, San Antonio, TX 78284. Colony stimulating factor 1 receptor (CSF1R) is a 150 kd transmembrane glycoprotein with intrinsic tyrosine kinase activity. CSF1R is important for survival and differentiation of mononuclear phagocytes, and may play a role in placental development. The extracellular ligand binding domain, and at least four regions of the cytoplasmic domain, are believed to regulate receptor function. To investigate the roles of CSF1R in cell proliferation and in regulating cellular metabolism, we have introduced mutations into two postulated regulatory regions of the cytoplasmic domain of CSF1R by oligonucleotide-mediated mutagenesis. Three in-frame deletions (glycine 684-leucine 750; glycine 684-serine 713; lysine 700-arginine 727) were introduced into the insertion sequence, a hydrophilic region of approximately 73 amino acids that interrupts the kinase domain. Tyrosine residue 561, in an analogous position to a regulatory tyrosine of insulin receptor was changed to phenylalanine. Mutated cDNAs have been inserted into retroviral expression vectors and are being transfected into mouse NIH3T3 cells to assess the biological activity of the mutant proteins. Some of the questions we are addressing include: Do the mutant CSF1R proteins retain tyrosine kinase activity?; Will they stimulate expression of "early" genes associated with growth factor induced cell proliferation?; Will they stimulate DNA synthesis? The studies should provide information on the mechanisms of growth factor receptor induced cell proliferation and are relevant to understanding deficits in growth factor receptor regulated pathways that occur during aging.

CG 107 AGE AND THE CARBOXYLMETHYLATION OF HUMAN BRAIN MYELIN BASIC PROTEIN, O.Z. Sellinger and M.F. Wolfson*, Lab. Neurochem., Mental Health Res. Inst., Univ. Michigan, Ann Arbor, MI 48109

The carboxylmethylation of a protein is a step in its partial repair, which involves the recognition and methylation by protein carboxylmethyltransferase II (PM II) of protein L-isoaspartate (isoAsp) residues, the cyclization of isoAsp methyl ester to an imide and the opening of the imide ring with the formation of varying proportions of damaged, isoAsp- and normal, Asp-containing molecules. Both Asp and asparagine (Asn) function as "abnormal aspartate generating residues" (AAGR). Yet, because Asn-derived isoAsp is repaired into Asp and not into the original Asn, slightly damaged proteins arise and are likely to accumulate in the aging cell in which the repair apparatus is presumed to falter.

As part of a project investigating the role of carboxylmethylation in the repair of myelin basic protein (MBP) during aging, we have carboxylmethylated MBP purified from human brains, aged 24-82 years. To assess the effects of in vitro treatments, known to increase the carboxylmethylation of other proteins, we heated MBP at 100°C for 120 min or exposed it to pH 10.5 at 37°C for 90 min. Heating converts Asp to isoAsp, while base converts Asn to isoAsp. Probably because MBP contains 2 Asn and 9 Asp residues, heating increased its carboxylmethylation much more than did exposure to base. Yet, an age study of the carboxylmethylation of base-treated MBP, aged 24-82 years, showed it to be age-dependent, with MBP (24) being about 66% more effective as a substrate for PM II than MBP (82), over a 6-fold range of MBP concentrations (2-13.5 μM). The age-dependent response of human brain MBP to the challenge of an alkaline treatment, with the youngest sample exhibiting the greatest response, implies significant differences in the AAGR of MBP as a function of age.

Supported by a grant from the National Institute on Aging.

CG 108 TWO DIMENSIONAL DNA GEL ELECTROPHORESIS: A NEW STRATEGY FOR ANALYSIS OF GENOME CHANGES DURING DEVELOPMENT AND AGING, L.J. Wangh and J.A. Sanchez, Department of Biology, Brandeis University, Waltham, MA 02254

Changes in genome structure and modification take place during vertebrate cell differentiation, maturation, and senescence. Tissue-specific patterns of DNA methylation are known for many individual genes, but the extent of methylation changes in whole genomes undergoing development is not clear. Senescent cells display an overall reduction in methylation, as well as other structural changes. But identification of the affected sites, or even characterization of possible age-dependent patterns of gene alteration has been hampered by the shear complexity of these genomes, as well as the stochastic nature of the aging process. We are evaluating high-resolution two-dimensional DNA gel electrophoresis as a method for surveying whole genomes or genome subsets in search of patterned changes in gene structure or modification. This approach, which uses two rounds of restriction enzyme digestion and electrophoresis, allows simultaneous detection of multiple changes without prior knowledge of the altered sequences. Two dimensional Southern blots can be probed for randomly dispersed moderately repeated sequences. The resulting spot patterns can then be analyzed using a new computer based scanning technology, Masterscan, from CSPInc. This approach is currently being applied to genomes having complexities of 10^6-10^9 base pairs. Supported by NIH DK31695, BRSG SO7 RR07044, and CSPInc.

Molecular Physiology

CG 200 IMMUNOSENESCENCE: AGE-RELATED ALTERATIONS IN TRANSMEMBRANE SIGNALLING SYSTEMS IN SPLEEN CELLS MAY RELATE TO IMMUNE DYSFUNCTION, Elliott J. Blumenthal and Alvin M. Malkinson, Molecular and Environmental Toxicology Program, University of Colorado School of Pharmacy, Boulder, CO 80309

Senescence represents a terminal stage of cell differentiation, and senescent lymphocytes have been shown to exhibit diminished responses to antigenic/mitogenic stimulation. Such responses may be mediated through second messengers such as Ca^{2+}, cAMP, and inositol phospholipids. We examined spleen cells from senescent mice for changes in transmembrane signalling systems. cAMP-dependent protein kinase (Pk-A) activity decreased in spleen cells obtained from 24 month old mice. This decreased activity correlated with decreased photoincorporation of 8-N_3[^{32}P]cAMP into membrane-associated Pk-A regulatory (R)-subunits and with increased labeling of a Mr=37,000 protein which is a partially degraded form of Pk-A R-subunits that has different biochemical properties. Ca^{2+}/phospholipid-dependent (Pk-C) activity also decreased in an age-dependent manner, exhibiting significant alterations by 6 months of age, an earlier time than Pk-A activity changes. Both the specific activity and the tissue concentration, as determined by immunoblotting analysis, of Pk-C decreased in older mice. The decreases in both Pk-A and Pk-C activities were generally greater in the membrane fractions than in the soluble fractions of these cells. An age-dependent increase in membrane-associated proteolytic activities was observed that could account for these alterations in kinase activities. We hypothesize that one reason for diminished immune responsiveness in senescent animals is an increase in proteolytic activity which causes degradation of Pk-A R-subunits and Pk-C that results in subsequent loss of cAMP-dependent and Ca^{2+}/phospholipid-dependent kinase activities. (This work was supported by USPHS grants ES-02370 and HL-37718, the Milheim Foundation, the Colorado Heart Association, and by RCDA CA-00939).

CG 201 INCREASED SUSCEPTIBILITY TO SV40 TRANSFORMATION WITH DEVELOPMENT AND in vitro AGING. Takahiro Kunisada, David B. Danner, and Edward L. Schneider, Laboratory of Molecular Genetics, National Institute on Aging, 4940 Eastern Ave, Baltimore, MD 21224. The incidence of most cancers increases with aging. To examine whether this increased risk might be related to a higher susceptibility of older cells to neoplastic transformation, we transfected rat fibroblasts aged in vivo and in vitro with origin defective SV40 DNA and measured the number of transformed foci. Substantial increases in the number of transformed foci were observed in cells from adult rats when compared with cells from embryos or weanlings. Much higher numbers of foci were also obtained at late passage, when 68% or more of the in vitro lifespan had been completed, while no foci were produced from cells at early or middle passage. To control for a possible age-dependent increase in uptake, integration, or expression of exogenous DNA, parallel cultures were transfected with a G418 resistance gene. The number of G418 resistant colonies did not increase with aging and, in fact, decreased in late passage embryonic cell cultures. Increased susceptibility to SV40 transformation appears to be a feature of development and in vitro aging in the rat.

CG 202 THE REGULATION OF EXPRESSION OF THE TRANSFERRIN GENE IN BRAIN-DERIVED CELL LINES. Kathryn Fischbach, Funmei Yang, Barbara H. Bowman, and Gwen S. Adrian, Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio, TX 78284.

Transferrin, an iron-binding glycoprotein, is synthesized in liver, brain, and other tissues. It functions to supply iron to cells for synthesis of iron-containing proteins. Synthesis of transferrin in nonhepatic tissues probably contributes to the homeostasis of cells in tissues that are separated by blood barriers from systemic circulation. Accordingly, in the brain, oligodendrocytes and choroid plexus synthesize transferrin. Iron in the brain increases with age. Although the functions of transferrin in the brain are poorly understood, the modulation of transferrin expression may play an important role in iron accumulation with age. We have chosen to approach this problem by studying the expression of transferrin in human glioma cell lines. Immunohistochemical studies indicate that these cell lines are of oligodendrocyte origin. Transferrin mRNA and protein synthesis has been shown by immunoprecipitation and Northern blot analysis. We have transfected the cell lines with a series of plasmids containing portions of the transferrin 5'flanking region fused to the CAT reporter gene. Sequences required for cell-specific expression, negative and positive modulation, and iron-level regulation are being studied.

CG 203 CHARACTERIZATION OF BRAIN FERRITIN. J. Fleming and J.G. Joshi. Department of Biochemistry, University of Tennessee, Knoxville, Tennessee 37996-0840.
Human brain ferritin (HBF) binds aluminum (Al) in vivo and in vitro and differs from other tissue ferritins in mobility on non-denaturing electrophoretic and isoelectric focusing gels (Fleming and Joshi PNAS, 84 7866-7870, 1987). We have further characterized HBF and rat brain ferritin (RBF) with respect to the effect of Al on iron (Fe) loading and release and subunit composition. Al causes a concentration dependent decrease in the initial rate of Fe(II) loading into demetallo-apo HBF but does not change the rate of Fe release from the corresponding holoferritin. HBF displays two subunits on SDS-PAGE Mr 22,000(H) and 19,000(L) that show immunological reactivity with anti-human (H) and (L) chain antibodies respectively. Unexpectedly HBF displays considerably more heterogeneity in the (H) chain than the (L) chain on reverse phase HPLC. Amino acid analysis of two homogeneous (H) chain peaks suggest that they are identical. Comparison of the amino acid composition of HBF (H) and (L) chains with the respective human liver ferritin (HLF) subunits suggests that while they are quite similar, the HBF (H) chain has fewer lys and the HBF (L) chain has more glu (or gln) and leu compared to the respective HLF subunits. RBF displays three subunits on SDS-PAGE Mr 23,00(H), 22,000(M) and 20,000(L). While the (L) chain shows reactivity with anti-human (L) chain antibodies, the (H) and (M) chains shows no reactivity with anti-human (H) chain antibodies.
Supported by the Robert and Monica Cole Neuroscience Foundation and by the Council for Tobacco Research.

CG 204 A METHYL VIOLOGEN (PARAQUAT)-SENSITIVE MUTANT OF CAENORHABDITIS ELEGANS,Naoaki Ishii, Kiyoko Takahashi, Satoru Tomita*, Tetsuo Keino** and Kenshi Suzuki, Department of Molecular Biology,Tokai University School of Medicine, Isehara, Kanagawa 259-11,Japan,*Showa University, Japan,**Celsi Cosmetics Co.,Japan.
There is an attractive idea that active oxygen species act as a promoter of aging. In order to get knowledge on the relationship between active oxygen species and aging, we isolated mutants of C. elegans which are hypersensitive to paraquat which is known to yield superoxide anion.
One of these mutants, mev-1, is about four times more sensitive to this drug than the wild type and also more sensitive to exposure to oxygen gas and to Cr. Mev-1 was found to have a shorter life span as compared with that of the wild type. Namely, the half-life for mev-1 was 8 days at 20°C, while that for the wild type was 12.5 days. It was found that the level of superoxide dismutase (SOD) of mev-1 was about half the level of the wild type.
It is suggested that activity of superoxide anion in normal cell is delicately controlled by SOD, however the exceeding superoxide anions or lack of SOD must cause cell damages and reduce the life span.

CG 205 AGE-RELATED CHANGES OF UBIQUITIN CONJUGATING CAPABILITIES IN THE EYE LENS, Jessica H. Jahngen and Allen Taylor, Laboratory for Nutrition and Cataract Research, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington St., Boston, MA 02111
Conjugation of ubiquitin (Ub) to proteins results in regulation of diverse processes, including intracellular degradation of altered and/or obsolete proteins. In the aged or cataractous lens, there is extensive accumulation of damaged proteins. Thus, it is useful to evaluate the processes involved in the accumulation rather than the timely degradation of lens proteins. Supernatants of actively dividing epithelial tissue from donors of 1 day to 94 years show considerable similarity of high molecular mass (HMM) endogenous Ub conjugates. Total cellular proteins from cultured human lens epithelial cells (HLEC) from fetal to 73 year old donors reveal Ub conjugates of >45 kDa. In lens cortex and core (1 day to 94 years, 6 cataractous), there is extensive age-related postsynthetic modification of soluble and insoluble proteins that occurs with increasing age. This indicates the possibility of a large protein pool for Ub conjugation. There are elevated levels of free Ub in lens cortex and core with advancing age. Substantially more Ub conjugates are found in lenses from older individuals. The majority of soluble Ub conjugates are in the 17-45 kDa range whereas insoluble Ub conjugates are primarily of HMM. It is plausible that changes in processes leading to the accumulation of Ub conjugates in cataract are similar to that seen in neurodegenerative disease (V. Manetto et. al., PNAS, 1988, 85:4501).
Supported in part by USDA Contract no 53-3K06-5-10, the Daniel and Florence Guggenhein Foundation, and the Massachusetts Lions Eye Research Fund, Inc.

CG 206 REPLICATION CONTROL AND DIFFERENTIAL GENE EXPRESSION IN AGING YEAST, S. Michal Jazwinski, James B. Chen and Neil E. Jeansonne, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112 The budding yeast *Saccharomyces cerevisiae* has a finite lifespan measured by the number of times the cell divides. As the cells became older, their generation times increased. The generation times of the daughter cells, during their first cell cycle, were virtually identical to that of their mothers. However, within three divisions, the daughters began dividing at the rate characteristic of young cells. These results indicate that the senescent phenotype is dominant in yeast, and that it is determined by a cytoplasmic factor produced by old cells and turned over in young cells. Lifespan determinations on *cdc7* mutant cells suggest that events at the G_1/S boundary of the cell cycle may be crucial for progress through the lifespan. Preparations of a DNA-replicative complex from yeast cells contained a *CDC7*-dependent protein kinase activity that phosphorylated an endogenous 48-kDa protein. This raises the possibility that phosphorylation of the replication machinery at the G_1/S boundary may play a role in cell cycle traversal and in coordination of successive cell cycles during the lifespan. In order to identify proteins involved in this regulation, a search for genes that are differentially expressed during the yeast lifespan was instituted. Young and old cells were prepared as a source of mRNA to generate specific cDNA probes. With these probes, six distinct genes that were preferentially expressed in either young or in old cells were cloned. The expression of at least two of these genes was not cell cycle-dependent, indicating that they are senescent-specific and not simply quiescent-specific.

CG 207 FROM GENES TO GENE PRODUCTS: REGULATION OF PROTEIN SYNTHESIS DURING AGEING AND TRANSFORMATION OF HUMAN CELLS, Suresh I.S. Rattan, Jens Cavallius, Bent Riis, Anastassia Derventzi and Brian F.C. Clark, Department of Chemistry, Aarhus University, DK-8000 Aarhus-C, Denmark. Even if there are genes for ageing, gerontogenes, it is the proteins coded by them that are the most likely molecules carrying out their instructions. Therefore, it is important to study the regulation of protein synthesis in order to understand the molecular basis of ageing. Slowing-down of protein synthesis during ageing is attributed to inefficient elongation factors (EF). Previously, we have reported cell cycle-, age-, transformation- and heat shock-related changes in amounts of active EF-1α, in its catalytic activity and in its RNA levels in normal and transformed human fibroblasts. Recently, we have observed a decrease in the amount of ADP-ribosylable EF-2 in growth-arrested human cells. Several means are being developed by us in order to modulate gene expression and the activities of elongation factors at the levels of transcription, translation and post-translational modifications for a better understanding of the regulation of protein synthesis during ageing.

CG 208 CALORIC RESTRICTION POSTPONES AGE-DEPENDENT DECLINES IN RAT LIVER MICROSOMAL MONOOXYGENASES, Douglas L. Schmucker and Rose K. Wang, Cell Biology & Aging Section, Veterans Administration Medical Center, Department of Anatomy and the Liver Center, University of California, San Francisco, CA 94143

Caloric restriction (CR) extends lifespan and retards the onset of physiological changes and pathologies associated with aging, but the underlying mechanisms remain unresolved. Richardson et al. recently demonstrated that age and dietary restriction-induced changes in the expression of a liver protein, α_{2u}-globulin, are elicited at the level of transcription (Richardson et al., 1987). Similar studies using well-characterized hepatic proteins or enzymes are essential. The male rat liver microsomal monooxygenases (MO's) constitute a well-characterized enzyme system whose age-related alterations are documented (see Schmucker, 1985 for a review). Our data demonstrate for the first time that CR significantly postpones or precludes age-related declines in the activity and microsomal concentration of liver MO's in male rats. In fact, several MO's exhibited marked enhancement in very old CR animals in comparison to young full-fed (FF) rats. However, the concomitant sex-dependent shifts in the distribution profile of cytochromes P-450 that occur during aging in FF male rats remain unaffected by CR and appear to parallel serum testosterone levels.

CG 209 ACCUMULATION OF OXIDIZED PROTEINS DURING AGING, Pamela E. Starke-Reed and Cynthia N. Oliver, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, U.S.A.
We have demonstrated that oxidized proteins accumulate during aging in several different models of aging. In extracts of hepatocytes from rats of various ages the levels of oxidized proteins increase progressively from 3 to 26 months of age with the largest incremental increase between 20 and 26 months. At least two liver enzymes, glutamine synthetase (GS) and glucose-6-phosphate dehydrogenase (G-6-PD), exhibit decreased specific activity and increased thermal lability without loss of immunological cross reactivity. These changes closely resemble changes observed when purified GS and G-6-PD are oxidatively inactivated by treatment with metal catalyzed oxidation (MCO) systems *in vitro* suggesting that GS and G-6-PD are oxidatively inactivated by MCO reactions *in vivo*. Because *in vitro* studies have demonstrated that MCO oxidized proteins are more susceptible to proteolysis by trypsin and subtilisin as well as by a cytosolic alkaline cysteine protease which exhibits a high degree of selectivity for the degradation of oxidized proteins compared to native proteins, the levels of alkaline protease activity was determined using oxidized protein substrates. The results indicate that hepatocytes from old animals possess only 20% of the alkaline protease activity of hepatocytes from young animals. Fractionation of these extracts using DEAE HPLC chromatography yielded nine fractions of protease activity but only two or three progressively decline with age. We are investigating the possibility that one or more of these peaks are related to the high molecular weight alkaline cysteine protease. Taken together the results suggest that during aging proteins are oxidized by MCO reactions and these altered forms accumulate in part because the proteases which degrade them are deficient or defective.

CG 210 ENHANCED CELL DIVISION AND PROTECTION AGAINST UV-INDUCED DAMAGE PROVIDED BY CELL EXTRACTS RICH IN GLYCOSPHINGOLIPIDS, James N. Thompson, jr., Department of Zoology, University of Oklahoma, Norman, OK 73019. Some rare membrane lipid components have been implicated in the protection of cells against damage by ultraviolet radiation, although the precise mechanism by which this occurs is not well understood. Glycosphingolipids (GSLs) from cell membranes have been found to stimulate replication when added to cell cultures. To test the possible relationship between enhanced cell division and UV protection or repair processes, controlled cultures of the ciliate protozoan Tetrahymena thermophila were treated with mammalian cell extracts enriched with glycosphingolipids and related membrane components. The experimental design allowed comparison of growth rates and UV-induced cell damage in control cultures with that in cultures given pre-UV and/or post-UV treatments. Several tested cell extracts that differed in GSL makeup were found to stimulate a rapid increase in cell division, and brief pre-treatments (e.g., 10 minutes) provided significant protection against UV damage as measured by cell survival after irradiation at 254 nm. Similar studies of cell division and repair are also being carried out on Drosophila melanogaster of different developmental ages. The possible role of these membrane lipid components in cell repair and replacement will be discussed. (Supported by grant from the Christiaan N. Barnard Foundation, Clinic La Prairie, Montreaux)

CG 211 ISOLATION AND CHARACTERIZATION OF CELL LINES FROM XERODERMA PIGMENTOSUM (COMPLEMENTATION GROUP D) FIBROBLASTS WITH DELETIONS AT THE HPRT LOCUS, Carla M. Wood and Robb E. Moses, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
Cell lines containing HPRT gene deletions have been derived from SV40 DNA-transformed fibroblasts of a male patient with xeroderma pigmentosum complementation group D (XP-D). To increase the frequency of mutations at this locus, the aneuploid parental cell line, MH3-XPD, was mutagenized with two agents known to interact with genomic DNA via distinct mechanisms, ethyl methanesulfonate (EMS) and bleomycin (BLM). During the course of these studies, the parental XP-D line was shown to contain a single copy of the HPRT gene, indicating that the cells remained physically hemizygous for this locus after the SV40 immortalization process. The HPRT enzyme activity from the initial 6-thioguanine-resistant ($6TG^r$) colonies was found to be only 50% of that observed for wild-type controls. Subcloning or repeat mutagenesis followed by continued maintenance in elevated concentrations of 6TG (30 to 60 μM) produced homogeneous cell populations with negligible growth in counter-selection medium (HAT). Unmutagenized cell cultures did not give rise to HPRT-deficient clones. Molecular analysis of the HPRT mutations in five clones with undetectable HPRT activity showed that four had large deletions. Three EMS-derived mutants fell into two classes: a putative point mutation or partial gene deletion and two total gene deletions. Two BLM-generated isolates from the same mutagenesis experiment were both found to have an intragenic deletion of approximately 28 kb beginning within the first intron near exon 1 and ending within the fourth intron near exon 4. This intragenic deletion cell line and the complete gene deletion lines will be of value both in examining intermolecular homologous recombination and as recipients for foreign gene expression.

Gene Regulation; Cell Cultures, Organelles and Viruses

CG 300 **EVIDENCE OF MITOCHONDRIAL INVOLVEMENT IN SCRAPIE INFECTION,** Judd M. Aiken, Judy L. Williamson and Richard F. Marsh, Dept. of Veterinary Sciences, University of Wisconsin, Madison, WI 53706
Scrapie is a transmissible, degenerative disease of the central nervous system. It occurs naturally in sheep and goats and has been introduced experimentally in hamsters, mice and rats. In spite of intense research effort the cause of the disease remains obscure. We have found mitochondrial nucleic acids to be preferentially associated with highly infectious scrapie preparations. This finding led us to consider a possible involvement of mitochondria in scrapie infection. Mitochondria were purified by repeated sucrose gradient centrifugation and found to contain high infectivity. Removal of mitochondrial outer membrane by osmotic shock or digitonin treatment resulted in no loss of titer. Submitochondrial particles prepared from purified mitochondria were also found to contain high infectivity. Examination of purified mitochondria by Western blot analysis using a monoclonal prion antibody indicated no contamination with scrapie-associated fibrils (SAF) or prions.

CG 301 ANALYSIS OF EUKARYOTIC GENE EXPRESSION *IN SITU* USING A REPORTER GENE ACTIVATED BY CHROMOSOMALLY-LOCATED TRANSCRIPTIONAL AND TRANSLATIONAL SIGNALS, Daniel G. Brenner, Sue Lin-Chao and Stanley N. Cohen, Stanford University, Stanford, CA 94305. In order to identify and study the expression of cell cycle-regulated genes as well as those involved in cellular senescence, a series of vectors (SIN/*lac*) has been constructed to allow activation of a reporter gene carried by a retrovirus upon integration of the virus into an expressed region of the chromosome. The retroviral-based system is designed to fuse the *lac* gene to chromosomally-located promoters, thus serving as an *in vivo* probe for regulatory sequences. The choice of the *E. coli lacZ* gene as the reporter also allows the formation of enzymatically-active *in vivo* fusions of β-galactosidase (β-gal) with eukaryotic proteins, and hence the analysis of translational, as well as transcriptional, control. Self-inactivating (SIN) retroviruses that lack both the enhancer and the promoter of the LTR have been employed as vectors to allow maximal expression of the reporter gene. The expression of β-gal activity in cos-7 cells infected transiently by SIN/*lac* constructs carrying a known promoter adjacent to the disabled retroviral LTR has demonstrated the validity of the approach. Helper cell lines producing the otherwise cryptic SIN/*lac* viruses have been cloned by using the polymerase chain reaction (PCR) to detect the viral RNA in the supernatant of the clones. Live cells have been isolated using a fluorescence-activated cell sorter for the phenotypic expression of β-gal and then subsequently screened for the retroviral sequences by PCR analysis. Additionally, co-cultivation of SIN/*lac*-producing Psi-2 cells together with mouse NIH3T3 cells has resulted in the activation of β-gal expression in the target cells. Experiments currently are in progress to investigate the utility of this technique in the isolation of genes induced by serum starvation of mouse NIH3T3 cells.

CG 302 LYMPHOCYTE DEATH BY OXIDATIVE STRESS AND AGEING: EFFECT OF INHIBITORS OF ADP-RIBOSYL TRANSFERASE (ADPRT). Andrea Cossarizza, Daniela Monti, *Maria Zannotti and Claudio Franceschi, Institute of General Pathology, University of Modena and *Institute of Histology and Embriology, University of Bologna, Italy. One of the hypothesized mechanism of programmed cell death is DNA fragmentation and a concomitant activation of ADPRT. Human quiescent lymphocytes exposed to xanthine oxidase-hypoxanthine, an enzymatic system which produces oxigen free radicals (OR), show a dose-dependent decrease of viable cells and an inhibition of responsiveness to PHA, measured as ^{3}H-TdR incorporation after 3-5 days of culture. The presence of ADPRT inhibitors, such as 3-aminobenzamide, during the damaging period (1 hour) prevents all these damages. We will present data obtained in 11 young and 11 old subjects and in 22 Down's syndrome (DS) subjects of different ages showing that: (i) lymphocytes from old subjects and from old DS (45-55 years old) were severely damaged by low concentrations of OR which had no effect on lymphocytes from young subjects; this damage was not prevented by 3-ABA; (ii) higher concentrations of OR decreased lymphocyte survival in all the groups, being such damage more evident in cells from aged and old DS subjects, in comparison with young donors and DS children; this damage was prevented by 3-ABA in all groups. In conclusion, the mechanisms which regulate cell death, and particularly those involving ADPRT, appear to be altered in lymphocytes from aged people and from subjects affected by a syndrome of accelerated ageing such as DS.

CG 303 TRANSFORMING GROWTH FACTOR BETA INDUCES SENESCENCE IN A RAT HEART ENDOTHELIAL CELL LINE, Gary R. Grotendorst[1], Kazuhiko Takehara[2], and Junji Matsuoka[1], (1)Dept. of Biology, Univ. of S. Fla., Tampa, FL 33620, and (2)Dept of Dermatology, Tokyo Univ., Tokyo, Japan. Transforming growth factor beta TGF-beta inhibits the growth of endothelial cells derived from human, bovine and rodent vessels. Exposure of the cells to TGF-beta for one hour is sufficient to completely inhibit the growth of the cells and to prevent the cells from responding to growth factors such as EGF. Long term exposure results in dramatic changes in cell morphology. After 5 to 7 days the cells have enlarged cytoplasms and exhibit an appearance similar to that of senescent cells. These changes in growth potential are accompanied by changes in the number of high affinity EGF receptors on the cell surface and on the expression of growth regulatory genes including c-fos, c-myc, and other growth factor inducible genes. Thus, the senescent phenotype may be the result of decreased production of certain growth regulated gene products.

CG 304 SULFATED GLYCOSAMINOGLYCANS IN AMYOTROPHIC LATERAL SCLEROSIS AND PARKINSONISM-DEMENTIA OF GUAM, ALZHEIMER'S DISEASE, DOWN'S SYNDROME AND CREUTZFELDT-JAKOB DISEASE, Don C. Guiroy, Alan D. Snow, D. Carleton Gajdusek, Richard Yanagihara, Ralph M. Garruto, National Institutes of Health, Bethesda, MD 20892 and University of Washington, Seattle, WA 98195

The mechanism by which amyloid fibrils are deposited in the nontransmissible and transmissible cerebral amyloidoses may involve the copolymerization of modified forms of the host precursor protein with glycosaminoglycans. Glycosaminoglycans are known to exert a direct effect on conformational protein folding. In Progeria increased carboxylated glycosaminoglycans has been convincingly shown to be related to the progression of the disease.

To determine whether glycosaminoglycans can be detected on the neuropathological lesions of the nontransmissible and transmissible cerebral amyloidosis, we stained formalin fixed brain sections with Alcian blue using the critical electrolyte concentration method of Scott and Dorling. By altering the molar concentration of magnesium chloride ($MgCl_2$) carboxylated and sulfated glycosaminoglycans could be stained selectively. Sulfated glycosaminoglycans were stained with $MgCl_2$ concentration above 0.2 M, and carboxylated glycosaminoglycans were stained when $MgCl_2$ were below 0.2 M.

Using 0.3 M and 0.7 M $MgCl_2$, the neurofibrillary tangles in parkinsonism-dementia and amyotrophic lateral sclerosis of Guam, Alzheimer's disease and amyloid plaques in Creutzfeldt-Jakob disease, Down's syndrome and Alzheimer's disease, and vascular amyloid deposits in Down's syndrome and Alzheimer's disease exhibited differential staining with Alcian blue indicating the presence of sulfated glycosaminoglycans in these diseases.

CG 305 CELLULAR SENESCENCE INVOLVES STOCHASTIC PROCESSES CAUSING LOSS OF EXPRESSION OF DIFFERENTIATED FUNCTION GENES, Peter J. Hornsby, Robert F. Ryan, Charles Y. Cheng, and Lian-Qing Yang, Dept. of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912

When grown for long periods in culture, bovine adrenocortical cells lose the expression of a differentiated function gene, steroid 17α-hydroxylase. Previously, we documented a decline in cyclic AMP-induced 17α-hydroxylase mRNA with increasing culture passage level. We used several methods to investigate the relationship of the loss of replicative capacity to the decline in 17α-hydroxylase expression. First, <u>in situ</u> hybridization with cDNA for 17α-hydroxylase after cyclic AMP induction showed that the previously observed decline in overall 17α-hydroxylase mRNA levels results from a decline in the fraction of cells hybridizing in the culture. In both mass and clonal populations cells that do not express 17α-hydroxylase are continually derived by a stochastic process from cells originally expressing the gene. Second, the cells' replicative potential was extended by transfecting them with cloned SV40 virus. Cells from a senescent subclone that were transfected expressed T antigen and were apparently immortalized. Induction of mRNA for 17α-hydroxylase by cyclic AMP was absent in this line of cells, as it was in the senescent cells prior to transfection. Early passage cells transfected with SV40 showed high levels of expression of 17α-hydroxylase. Thus, T antigen expression selectively affects growth, but preserves the state of expression of a differentiated function gene as it was prior to transfection. Third, experiments with combined bromodeoxyuridine labeling and <u>in situ</u> hybridization show that there is no association between the replicative status of individual cells and their 17α-hydroxylase expression. However, experiments in which cells were prevented from division by mitomycin C show that replication is necessary for the 'switching' in gene expression to occur.

CG 306 MOLECULAR AGING MODELS. Marguerite M. B. Kay, G. Bosman, G. Johnson, Department of Medicine, Texas A&M Univerity, Veterans Center, Temple, TX.

As part of our ongoing studies on mechanisms of cellular aging, we searched for models of accelerated and decelerated cellular aging. We anticipated that such models would allow us to dissect molecular aging and provide insight into mechanisms. Initially, we investigated models for aging in vitro. The validity of these treatments as model systems for RBC aging was evaluated using a "RBC aging panel" which provides a biochemical profile of a senescent red cell: 1, increased breakdown of band 3 as detected by immunoblotting; 2, decrease in anion transport efficiency as detected with a sulfate self-exchange assay; 3, decrease in total glyceraldehyde 3-phosphate dehydrogenase (G3PDH) activity with an increase in membrane-bound activity; and 4, increase in the binding of autologous IgG as detected with a protein A-binding assay. Neither incubation with the free radical-generating xanthine oxidase/xanthine system, nor treatment with malondialdehyde, an end product of free radical-initiated lipid (per) oxidation, results in age-specific changes. Loading of the cells with calcium, and oxidation with iodate results in increased breakdown of band 3, but does not lead to increased binding of autologous IgG. Only RBC that have been stored for 3-4 weeks show the same structural and functional changes as observed during aging in vivo. A search for "experiments of nature" that might provide insights into the process of normal cellular aging revealed that neither glucose 6-phosphate dehydrogenase deficiency (G6PD) nor hemoglobin Köln cells exhibited characterists of accelerated cellular aging. During our investigations we discovered 2 mutations and/or clinical alterations of RBC band 3. One mutation, high molecular weight band 3, results from an addition and to the transmembrane, anion transport region of band 3. A second band 3 alteration is characterized by accelerated cellular aging as determined by a "RBC aging panel," and cellular removal. The patient's reticulocyte count is ~20% indicating the destruction and replacement in situ of 20% of circulating red cells daily. We gave this band 3 alteration the descriptive name "fast aging" band 3 because these cells exhibit all the characteristics of old RBC. We suspect that "fast aging" band 3 is more susceptible to proteolysis than is normal band 3.

CG 307 ALTERED FIBRONECTIN mRNA LEVELS DURING CELLULAR SENESCENCE IN VITRO, Don A. Kleinsek, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030.

Normal cells in culture exhibit a finite replicative lifespan *in vitro*. Previous work indicates that this process is genetically programmed. To understand the molecular events responsible for this cellular senescence, gene expression changes between young, proliferation competent and senescent human diploid fibroblast cells (HDF) is assayed for. This is accomplished by the differential screening of a senescent cDNA library with young and senescent cDNA probes. Less than 0.05% of the recombinants from the lambda vector show a senescent-specific expression. The senescent-specific cDNA clones characterized hybridize to a mRNA size of 7.8 kilobases. The sequence of these cDNAs is homologous to the 3' terminal portion of human fibronectin. The steady-state mRNA level for this glycoprotein is a function of the growth state of the HDF cells. Thus, young, proliferating cells produce low levels of the mRNA whereas cells that are serum or cell-density arrested or senescent have high up-regulated levels of the fibronectin transcript. This increase in message level for fibronectin is not due to an amplification or rearrangement of the fibronectin gene. However, a fibronectin sequence variant produced during senescence may account for the high fibronectin mRNA level.

CG 308 DNA POLYMERASE α CHANGES AFTER EXPOSURE TO PROTEIN KINASE C OR ALKALINE PHOSPHATASE, S.W. Krauss and S. Linn, Dept. of Biochemistry, Univ. of California-Berkeley, Berkeley, Ca. 94720

DNA polymerase α is a major participant in DNA replication and some types of repair. Previously we observed changes in α polymerases during aging which may be related to general processes occurring as cells enter into a non-replicative state. These studies were extended to assess effects of phosphorylation and dephosphorylation on polymerase α activity. Protein kinase C stimulates polymerase α activity, increases polymerization fidelity *in vitro*, and reduces the Km for DNA substrates but not for dNTPs. It also alters affinity of polymerase α for DNA-cellulose but has little effect on primase activity associated with polymerase α. No exonuclease activity can be detected in polymerase α fractions before or after protein kinase C exposure. A peptide of ~190 kD phosphorylated by kinase is specifically immunoprecipitated by anti-α polymerase. This may be the polymerase α catalytic unit observed to be phosphorylated *in vivo*. In contrast, alkaline phosphatase treatment reduces polymerase activity, fidelity, and binding to DNA-cellulose. These observations further delineate the role of protein phosphorylation in modulating DNA polymerase function and may ultimately relate to cell cycle events and aging.

CG 309 AGE-DEPENDENT POLIOMYELITIS OF MICE (ADPM): INTERACTION BETWEEN ENDOGENOUS RETROVIRUS AND EXOGENOUS TOGAVIRUS IN THE DISEASE.
Peter G.W. Plagemann, Christopher H. Contag and John T. Harty, Department of Microbiology, University of Minnesota, Minneapolis, MN 55455

ADPM is induced in C58 and AKR mice by an acute infection with lactate dehydrogenase-elevating virus (LDV). Susceptibility of these mice to ADPM is genetically linked to the presence of proviral copies of an N-tropic, ecotropic murine leukemia virus (MuLV) and the permissiveness for the replication of this virus. The susceptibility increases with increasing age of the mice and after X-irradiation or treatment with cyclophosphamide. Destruction of anterior horn motor neurons in paralyzed mice is due to the cytocidal replication of LDV in these neurons. Susceptibility of the neurons to LDV infection correlates with and seems to be dependent on the expression of the endogenous MuLV in these neurons, which is specifically increased 10-100 fold with increasing age of C58 mice and after cyclophosphamide treatment and X-irradiation. Passive immunization of C58 mice with antibodies to the glycoprotein of LDV (VP-3) protects the neurons from LDV infection without affecting the replication of LDV in macrophages, its normal host cell. ADPM resembles in many features amyotrophic lateral sclerosis of humans and thus may serve as model for this human neurological disease.

CG 310 COMPARISON OF SIGNALS REQUIRED BY T CELLS FROM YOUNG AND AGED MICE FOR UPREGULATION OF IgD RECEPTORS. C.D.Swenson, B. Xue, A.R. Amin, R.F. Coico, & G.J.Thorbecke. NYU Sch. of Med., NY, NY 10016 and CUNY Med. Sch., NY, NY 10031.
Incubation of T cells from young (yg) adult mice for 1 hour at 37°C with oligomeric IgD upregulates the expression of receptors specific for IgD (IgDR), as shown by rosetting with IgD-coated erythrocytes. Such $IgDR^+$ cells mediate augmenting effects of IgD on antibody production. T cells from aged mice fail to show an increase in IgDR even after overnight (ON) incubation and aged mice do not show immunoregulatory effects of IgD. Upregulation of IgDR by IL-2 with normal T cells requires ON incubation and is low but not absent with T cells from aged mice. Preincubation of T cells from aged mice with IL-2 for 2 hours renders them partially responsive to IgD. Cycloheximide inhibits upregulation of IgDR by IL-2 and IL-4 but not by IgD on normal splenic T cells. However, cloned T cells with receptors for IL-2 exhibit IgDR within 1-2 hours of exposure to IL-2 and de novo protein synthesis is not required for this upregulation of IgDR. Exposure of T cells from aged or yg mice for 1-2 hours to ionomycin (5μg/ml) and phorbol myristic acid (PMA,1ng/ml) also upregulates IgDR, whereas neither ionomycin nor PMA alone induces any change in IgDR. Forskolin (10-50 μM) also causes IgDR upregulation. The importance of a rise in intracellular Ca^{++} in IgDR modulation is also suggested by the observation that ionomycin, while not affecting upregulation by IgD of IgDR on cells from yg mice, enables cells from aged mice to increase their IgDR expression on simultaneous exposure to IgD. The possible roles of cAMP and protein kinase C activation in IgDR upregulation will be studied further. (Supported by AG-04860)

CG 311 IDENTIFICATION OF TRANSGENIC MICE CARRYING THE CAT REPORTER GENE WITH PCR, Christi A. Walter, Department of Cellular and Structural Biology, University of Texas Health Science Center San Antonio, San Antonio, TX 78284.

Identification of transgenic mice in an accurate and rapid manner is crucial to transgenic mouse experiments. DNA isolated from tail sections is typically used for diagnosis of transgenics by slot blot or Southern analyses. Here we report a polymerase chain reaction procedure to detect transgenic mice carrying the chloramphenicol acetyl transferase (CAT) gene with appropriate accuracy and rapidity.

Mouse tail DNA is prepared by standard procedures (Krumlauf et al., 1985) and a small aliquot combined with all four deoxyribonucleotides, reaction buffer, Taq polymerase, and primers that flank a region of the CAT gene. Thirty-five cycles of polymerization are performed to produce an intense 320 bp band in an agarose gel from transgenic DNA. Southern analysis of some DNAs subjected to amplification indicated the 320 bp band represents a region of the transgene.

Polymerase chain amplification is advantageous in several respects. First, use of radioactive compounds is substantially reduced. Second, the time required to identify transgenics is reduced. Third, because so little DNA is required for PCR there is genomic DNA available for subsequent Southern analysis of transgenics for integration information. Fourth, many different transgenic lines can be analyzed when using the CAT gene as a reporter gene. Fifth, single copy transgenics are reliably detected by amplification. Accurate, rapid and efficient identification of transgenic mice is possible with polymerase chain reaction amplification procedures.

CG 312 LIPOPROTEINS AND IMMUNE FUNCTION IN THE AGED, G.Wick, Institute for General and Experimental Pathology, Fritz-Pregl-Str. 3, 6020 Innsbruck, Austria

We have recently confirmed that the age-related decline in mitogen function correlates with a small, but significant, decrease in plasma membrane fluidity as assessed at the single cell level by flow cytometric analysis of the fluorescence polarisation of the probe diphenylhexatriene (DPH). Our working hypothesis is that cholesterol homeostatic regulation in lymphocytes from the elderly may be inefficient and thereby lead to an elevated membrane cholesterol/phospholipid molar composition with resultant decreased fluidity. We have shown that (a) T-cells from the elderly express higher levels of low density lipoprotein (LDL) receptor activity than those from the young; (b) that T-cells from the elderly express higher levels of high density lipoprotein (HDL) binding sites than those from the young. The physiological relevance of these binding sites is under investigation, with respect to reverse cholesterol transport and delivery of lipids to peripheral tissues; (c) that LDL receptor activity and HDL binding sites correlate positively at the individual level; (d) that LDL and HDL binding/uptake do not correlate with membrane fluidity or mitogen responsiveness. This is to be expected since uptake of cholesterol via these receptors is probably minimal compared to that via non-receptor mediated mechanisms under physiological conditions. Nevertheless, the altered receptor activity probably reflects faulty down-regulation, i.e. inefficient monitoring of cellular cholesterol levels. We are currently investigating whether cholesterol biosynthesis is also inefficiently regulated by comparing the HMG CoA reductase activity in cells from young and elderly blood donors. (Supported by the Austrian Research Council (project S-41/01) and the Austrian Ministery of Science and Research.)

CG 313 HUMAN α_2-HS-GLYCOPROTEIN GENE AND ITS EXPRESSION IN CHONDROCYTES

Chong-Chou Lee, Barbara H. Bowman, Zvi Schwartz*, Barbara D. Boyan* and Funmei Yang, Department of Cellular and Structural Biology and *Department of Orthopaedics, The University of Texas Health Science Center at San Antonio, Texas 78284. The α_2-HS-glycoprotein (AHSG) is a plasma protein reported to be important for bone mineralization and the immune response. It is synthesized mainly in the liver yet selectively concentrated in the bone matrix. The concentration of AHSG in the bone matrix appears to vary in different developmental stages. Fetal bone contains at least 10 times more AHSG than does adult bone, while neonatal bone contains 7 times more AHSG than adult bone. In recent studies by Ishikawa et al. and by our laboratory, AHSG was shown to increase chondrocyte alkaline phosphatase (ALPase) activity, indicating that AHSG is important in ALPase expression in normal growth plate cartilage differentiation. We have characterized the AHSG gene. We now report the use of the human AHSG cDNA as a probe to investigate the developmental and tissue-specific expression of AHSG. A 1.6 kb AHSG transcript, the same size as the full-length human AHSG mRNA, was detected in mouse and rat liver as well as stomach. Interestingly, a mRNA transcript of 2.3 kb hybridized with AHSG cDNA was detected in mesenchymal cells, resting chondrocytes and growing chondrocytes derived from rat, but not in a human osteosarcoma cell line. This raises the possibility that AHSG may also be produced in cartilage and that locally synthesized AHSG may be required for bone maturation. Since levels of AHSG vary in fetal, newborn and adult bone matrix, expression of the AHSG gene appears to be developmentally and tissue-specifically regulated. We are investigating the origin and the gene product of the 2.3 kb AHSG transcript detected in chondrocytes.

Cell Senescence and Death; Age-Related Diseases

CG 400 EFFECTS OF ONE YEAR OF MICRONUTRIENT SUPPLEMENTATION ON CELLULAR IMMUNE FUNCTIONS IN THE ELDERLY, John D. Bogden, James Oleske, Marvin Lavenhar, Elizabeth Munves, Francis Kemp, Kay Bruening, Kimberly Holding, Thomas Denny, Bart Holland and Donald Louria, Dept. of Preventive Medicine, UMDNJ-New Jersey Medical School, Newark, NJ 07103

There is general agreement that cellular immune functions decline with age. In addition, it is clear that severe malnutrition or severe deficiencies of single nutrients such as zinc can compromise cellular immune functions. However, most elderly people in the United States do not have severe nutritional deficiencies, and it is not known if nutrient supplementation can prevent the age-related decline in cellular immune functions in these individuals. The objective of this study was to determine the effects of a year of Zn supplementation on cellular immune functions in the elderly. Free-living subjects (n=63), aged 60-89, were given a placebo, 15 mg Zn or 100 mg Zn daily for 12 months. All subjects also received a multivitamin/mineral supplement that contained no additional Zn. Blood samples were drawn and immune functions and diets assessed prior to and at 3, 6, 12, and 16 months after beginning supplementation. Dietary folate, pyridoxine, alpha-tocopherol, copper, and zinc were consistently below recommended intakes. Natural killer cell activity was transiently enhanced by the 100 mg/day dose of Zn. There was a progressive improvement in delayed dermal hypersensitivity (DDH) and in lymphocyte proliferative responses to two mitogens in all treatment groups; this may have been due to one or more components of the multivitamin/mineral supplement administered to all study subjects. The enhancement of DDH was significantly greater in the placebo group than in either zinc treatment group. (Supported by NIH grant AG04612.)

CG 401 COLLAGEN PRODUCTION BY DERMAL FIBROBLASTS DURING IN VIVO AGING AND IN VITRO CELLULAR SENESCENCE, S.A. Bruce, A.M. Choi, K.G. Cook and S.F. Deamond, The School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, MD 21205.

Using the Syrian hamster (SH) as an experimental system, we are investigating the normal development and aging of dermal fibroblasts in vivo (proliferation, extracellular matrix (ECM) production, quiescence and reactivation during wound repair) to determine whether the in vivo differentiation program and mature function of these cells is related to their proliferation and senescence pattern in vitro. Preliminary electron microscope studies show that extracellular collagen accumulation begins at about the 13-14th day of gestation in this species and is complete by 1-2 months of age, after which time dermal fibroblasts appear quiescent in terms of both protein synthesis and proliferation. Primary cultures of SH 13 day gestation fetal dermal (FD13) fibroblasts exhibit an initial phase of logarithmic cell growth followed by a non-proliferative senescent state after ~25 population doublings (Mech Ageing & Devel, 34:151). Furthermore, continuous exposure of FD13 cells to all-trans retinoic acid reduces this proliferative life span in a dose-dependent manner. In both untreated and retinoic acid-treated cell populations, the level of procollagen α1[I] mRNA decreases during the last 10-20% of the in vitro proliferative life span as the cells enter the non-proliferative, senescent state. Thus, proliferation and collagen type I expression may be directly related in SH dermal fibroblasts both in vivo and in vitro, suggesting that there may indeed be parallels between the behavior of these cells in vitro and vivo. (Supported by NIH AG07875)

CG 402 RETINAL DEFECTS IN ALZHEIMER'S PATIENTS, Sandra C. Ebeling[1], Janet C. Blanks[1] and Robert H.I. Blanks[2], [1]Doheny Eye Institute, Department of Ophthalmology, University of Southern California School of Medicine, 1355 San Pablo St., Los Angeles, Calif. 90033, U.S.A., and [2]Department of Anatomy and Neurobiology, University of California Irvine, Irvine, Calif. 92717, U.S.A.
A recent report by our group (Hinton et al., N. Engl. J. Med. 315:485-487, 1986) demonstrated the involvement of the retina in the constellation of neurodegenerative changes present in Alzheimer's disease (AD). In addition, ganglion cell counts from retinal whole-mounts (Bassi et al., Soc. Neurosci. Abst. 1987) suggest loss of ganglion cells (GCs) throughout the retina. The present report further characterizes the visual defect in AD by examining the macular region of the retina in radial sections from 2 AD patients and 2 age-matched controls. GCs, glial cells and pyknotic nuclei were drawn, and digitized to determine cell number, diameter and area at several retinal eccentricities. Results indicate that there is a 46-84% loss of GCs in the macula of AD patients compared to controls. Many of the remaining GCs are pyknotic or show signs of degeneration. The greatest loss of GCs is within 3 deg. of the foveola and is primarily among the large GCs. Surviving GCs in the AD patient are smaller in diameter (13.3 $\pm$ 2.3 μm) than controls (16.6 $\pm$ 1.7 μm). Concomitantly, there is a 130-210% increase in glial cells in the GC layer of AD patients. More importantly, and as a control for cell shrinkage in histological preparation, the glia were the same diameter in AD patients (8.93 $\pm$ 2.1 μm) and controls (9.95 $\pm$ 1.9 μm). The present findings suggest that the lesion in AD may differentially affect the large GCs and may have a greater effect in centrally placed GCs of the macula. These data may provide the anatomical correlates for the visual deficits noted in AD patients.

CG 403 HUMAN FIBROBLASTS HAVE THE SAME PROLIFERATIVE CAPACITY WHEN CULTURED IN SERUM FROM YOUNG OR OLD COWS, *Junli Feng, Cheryl Wistrom, and Bryant Villeponteau, Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109.*
Diploid human fibroblasts have a limited proliferative capacity when grown *in vitro*, leading to senescence and eventual death of the culture. Results of earlier work suggested that serum from older animals might contain higher concentrations of growth inhibitors which could accelerate the onset of cellular senescence. To reexamine this question, we cultured human MRC-5 cells in fetal calf serum and in serum from cows of various ages. Surprisingly, we found that human MRC-5 cells, which are normally cultured in fetal calf serum, exhibit nearly identical proliferative capacity when grown in serum from young or old cows. Our failure to detect significant differences in the proliferative capacity of MRC-5 cells cultured in serum from cows of widely different ages suggests that the balance of growth-stimulating and growth-inhibiting factors in serum do not vary significantly as a function of animal age.

CG 404 TERMINAL DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION IN DIFFERENTIATING FIBROBLAST STEM CELLS OF BROWN-NORWAY RATS, Pal I. Francz, Klaus Kontermann, Jürgen Mollenhauer * and Klaus Bayreuther, Institut für Genetik, Universität Hohenheim, D7000 Stuttgart 70 and *Institut für Pharmakologie und Toxikologie, Universität Erlangen, D8520 Erlangen, F.R.G. BN-rat fibroblasts in in vivo-in vitro and in vitro cell culture systems differentiate in the mitotic compartment along a cell-lineage fibroblast MF I - MF II - MF III. If appropriate methods are applied, the fibroblast MF III shifts spontaneously to the fibroblast PMF IV. The postmitotic fibroblasts differentiate in the postmitotic compartment along the sequence PMF IV - PMF V - PMF VI. After a long period in stationary culture with a very high macromolecule synthesis, PMF VI degenerates by a genetic apoptosis programme. Out of the postmitotic fibroblast PMF VI, transformed cells T-MF arise spontaneously by a budding process. They show specific marker proteins in common. The transformed fibroblasts T-MF differentiate again along a cell-lineage T-MF I - II - III (mitotic) T-PMF IV - V - VI - VII (postmitotic). Out of the TF sequence, an additional cell-lineage originates after neoplastic transformation, the NT-F sequence NT-MF I - II - III (mitotic) NT-PMF IV - V - VI - VII (postmitotic). For the biochemical identification of the 21 fibroblast cell types 11 biochemical parameters (e.g., 2-d-gelelectrophoresis) have been analysed. Thus the normal cellular aging of the primary and secondary mitotic and postmitotic fibroblasts is a seven stage differentiation sequence of the fibroblast stem-cell system controlled by genetic programmes. The development of the transformed cells out of the postmitotic normal fibroblasts is a novel finding which could explain the increasing frequency of fibroblast tumours (sarcomas) as a function of the aging fibroblast stem-cell system in the aging organism.

CG 405 CHARACTERIZATION OF THE GENOMIC ORGANIZATION AND PEPTIDE DOMAIN OF THE ALZHEIMER'S DISEASE AMYLOID PRECURSOR, Edward M. Johnstone, Michael O. Chaney*, Robert Moore, Karen Ward, Franklin H. Norris and Sheila P. Little, Molecular Biology Department and Organic Chemistry Department*, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285. In order to better understand the processing of the Alzheimer's disease amyloid precursor protein, we have cloned and sequenced that region of the genome that codes for the A4 peptide. Two exons separated by a 5.5 kb intron define this region. Our characterization of the A4 peptide amino acid sequence shows similarity to the structure of soybean trypsin inhibitor (Kunitz). Our finding is distinct from the domain already described by Tanzi et al., Nature 331:528; Ponte et al., Nature 331:525; and Kitaguchi et al., Nature 331:530 (1988). Application of an algorithm (PEST) suggests that the PreA4 protein may be rapidly processed. Moreover, the exon organization, Kunitz domain duplication and transmembrane location of A4 suggest that PreA4 is similar to growth factor precursors and perhaps processed as such.

CG 406 Proliferative Responses of Aged Gut Mucosal T and B Cells. Intracellular Events. Hidenori Kawanishi, Mucosal Aging Research Lab. SUNY at Stony Brook and Northport VAMC, Northport, NY 11768. Gut mucosal immunosenescence is manifested by a decline largely in T cell and, less prominently, in B cell functions, similar to nonmucosal lymphoid tissues. Aging-related subcellular deficiencies are likely responsible for this decrease. Synergistic effects of protein kinase C(PKC) activator phorbol ester (PMA) and calcium ionophore ionomycin (Ion) were demonstrated in both aged T and B cells, but nearly at 6-7/10th levels of the young. Addition of IL 2 in the T cells restored the diminished proliferation. Con A-induced proliferative responses of aged T cells were impaired more profoundly than those of PMA and Ion. IL 2 did not entirely correct a decline in the Con A-stimulated T cell response of old mice. Proliferation of LPS-stimulated aged B cells was slightly diminished. PMA and/or Ion did not alter the activated B cells. IL 4 and IL 6 augmented the LPS-stimulated aged B cells to the level of LPS-stimulated young B cells. The results suggest that the age-associated gut mucosal immune decline is closely related to alterations in signal transduction and in the subsequent induction of intracellular biochemical regulation in T and B cells.

CG 407 ADVANCED GLYCOSYLATION ENDPRODUCTS ARE CHEMOTACTIC FOR NORMAL HUMAN MONOCYTES: ROLE IN AGING, Martina Kirstein and Helen Vlassara, Laboratory of Medical Biochemistry, The Rockefeller University, New York, NY 10021

Formation of advanced non-enzymatic glycosylation endproducts (AGEs) on long-lived tissue proteins and AGE-mediated protein crosslinking both increase with time and may irreversibly damage protein function in aging tissues. Recently a new macrophage receptor which specifically recognizes AGE-modified proteins has been described to mediate the removal of glucose-modified senescent macromolecules. We now demonstrate that AGEs also have chemotactic activity for normal monocytes, possibly participating in their recruitment from the circulation. AGE-modified protein was prepared by incubating bovine serum albumin (BSA) with 50 mM glucose-6-phosphate for 4 weeks at 37°C. In modified Boyden chambers, normal human peripheral blood monocytes were shown to move directionally to AGE-BSA (58% of positive control 10 nM FMLP), whereas unmodified albumin did not elicit such a response. Furthermore, human myelin, naturally glycosylated to high levels of AGE during the life of a 60-year-old diabetic individual, was 6 times more chemotactic for monocytes than was myelin from a one-month-old infant. Chemotactic activity of both *in vitro* prepared AGE-BSA and *in vivo* glycosylated diabetic myelin remained undiminished after enzymatic protein digestion, indicating that chemotaxis was due to AGE moieties rather than to intact protein structure. These data suggest that AGEs may act as chemotactic stimuli for the recruitment of circulating monocytes to sites of accumulated aged proteins where they trigger uptake and removal of senescent material and contribute to normal tissue homeostasis.

CG 408 SYNTHESIS OF UNIQUE PROTEINS DURING CELL DEATH, Richard A. Lockshin, Department of Biological Sciences, St. John's University, Jamaica NY 11439

Intersegmental muscles and labial glands of the tobacco hornworm, Manduca sexta, degenerate during metamorphosis. Protein content and synthesis from growing, static, and degenerating tissues has been analyzed, and RNA isolated and translated. The translation products were run on two-dimensional electrophoresis. Growing and static muscles synthesize predominantly myofibrillar proteins, and cytosolic proteins constitute a vanishingly small proportion of the proteins identified even by silver staining. When the muscle begins to degenerate, a large number of proteins is seen on the gels. Many of these are fragments of myofibrillar proteins, but over 30 can be recognized as new translation products. Most of the translation products are found in two regions, one ranging from 20 to 40 kDa and with a pI of 6.5-6.9; and the other approximately 50-70 kDa and pI of 5.8-6.2. The pattern is identical in two separate instances of degeneration. In the labial glands, during the early phases of degeneration, small heat shock proteins are among the few prominently synthesized. Once the gland collapses, as determined by histology and by failure of mitochondrial respiration, larger heat shock proteins and one or two others represent the entire synthetic activity of the gland.

CG 409 CHANGES IN PROTEIN TURNOVER AFTER HEAT SHOCK ARE RELATED TO ACCUMULATION OF ABNORMAL PROTEINS IN AGING DROSOPHILA MELANOGASTER, Aleksandra Niedzwiecki and James E. Fleming, Ryoichi Sasakawa Center for Aging Research, Linus Pauling Institute of Science and Medicine, 440 Page Mill Road, Palo Alto, CA 94306.

Adult Drosophila melanogaster kept at 25° show a progressive decline of protein synthesis and degradation rate with age. After exposure of young flies to 20 min. of heat shock at 37°,the incorporation of ^{35}S Methionine into TCA precipitable proteins decreases more than 60% in comparison to nonstressed flies. This decrease is accompanied by a lower protein degradation rate. The same stress in old flies results in 2-3 fold increase in protein synthesis as compared to non heat shocked flies of corresponding age or to young heat-shocked flies. Those flies also have faster protein turnover than unshocked controls. An effect similar to that observed in senescent Drosophila occurs in young flies when fed canavanine, an arginine analog, before and during heat shock. The difference in heat shock response between young and old insects can be related to accumulation of abnormal proteins and lower proteolytic activity with age.

CG 410 UV-INDUCED ORNITHINE DECARBOXYLASE IN HUMAN SKIN FIBROBLASTS AS A MARKER OF AGING, Niggli H.J., Cosmital SA, Research Company of Wella AG, Darmstadt (FRG), rte de Chésalles 21, CH-1723 Marly (Switzerland)

Deoxyribonucleic acid (DNA), the genetic material of all cells, figures prominently in many theories of aging. It is known that ultraviolet light (UV-light) leads to premature aging and the production of cyclobutane-type pyrimidine photodimers is the predominant photoreaction in the far-UV range. Induction of ornithine decarboxylase (ODC) as well as pyrimidine dimer formation have also been implicated in UV-induced mutagenesis and carcinogenesis. Additionally, ODC is involved in the synthesis of polyamines and may therefore participate in the modulation of gene expression. We review our studies on the importance of UV-light for the induction of ODC-activity and the formation of cyclobutane-type pyrimidine photodimers in human skin fibroblasts. Our results showing a correlation between ODC-response and pyrimidine dimer formation support the notion that DNA is the primary chromophore involved. Furthermore, we observed that ODC in fibroblasts from younger donors can be significantly more stimulated compared to older donors. In this respect it is interesting that the repair deficient XPA cells from a young donor lacked UV-stimulated ODC-induction. XP patients are said to manifest premature aging of the skin although published descriptions are lacking. Our data may point to a relationship between aging and this autosomal recessive disorder. A possible explanation for this connection may be, as shown by many sources, that the control of gene expression in higher organisms is related to the methylation of cytosine in DNA, and that the pattern of methylation is inherited. For instance, Steglich et al. provided data that ODC in some hamster cell-lines is inactivated by DNA methylation. Diminution of ODC-response in normal aging cells from adult donors and loss of ODC-induction in XP-fibroblasts is therefore the result of DNA methylation.

CG 411 ALTERATIONS OF INTERLEUKIN 2 RECEPTOR EXPRESSION IN THE AGED. FM Orson, CK Saadeh, DE Lewis, DL Nelson. Baylor College of Medicine & VA Medical Center, Houston, TX 77030; NCI, Bethesda, MD 20814. Aged individuals have depressed cell-mediated immunity and cellular proliferation. Since T cell growth depends on the expression and function of interleukin 2 (IL-2) receptors, we measured surface expression, IL-2R affinities, soluble IL-2R beta chain release, and beta chain specific mRNA production in vitro in PHA-stimulated cells from healthy aged ($\geq$65) and young ($\leq$40) donors. At 72h, the fraction of cells expressing IL-2R beta chain (anti-Tac positive) was lower in the aged group (44% vs 56%, $p<.05$). The mean numbers of high and low affinity receptors per Tac-positive cell were equivalent in cells from aged and young donors (1760 vs 1534 high affinity, and 22575 vs 18386 low affinity, $p>.2$). The affinities of each kind of receptor were not different. Despite the lower fraction of Tac-positive cells in old donor cultures, total RNA recovered at 24h contained an equal or larger proportion of IL-2R beta chain specific mRNA. Old and young donor cells also released equivalent quantities of soluble beta chain into culture supernatants. Although free beta chain binds IL-2, it did not inhibit proliferation by competing for IL-2. Saturating amounts of exogenous IL-2 did not increase the proliferative responses of cells in either young or old donor cultures. Furthermore, soluble "anchor-minus" IL-2R beta chain added at 10 fold higher concentrations than endogenous production did not suppress responses. Thus, despite production of beta chain IL-2R specific mRNA and release of soluble beta chain in normal or high quantities, a lower proportion of mitogen-stimulated peripheral blood lymphocytes from aged donors express surface IL-2R beta chain correlating with their diminished proliferation. NIA AG07068 and VA.

CG 412 CHARACTERIZATION OF AORTIC SMOOTH MUSCLE CELLS (SMC) IN CULTURE FROM DIABETIC RATS. Assunta Pandolfi, Sibylle Hess, Vilma L. Giandomenico, Maria Rita Milani*, Egidio Marchi*, Maria Benedetta Donati and Andreina Poggi, Istituto Mario Negri ,Consorzio Mario Negri Sud, 66030 S. Maria Imbaro and *Alfa-Wasserman Laboratories, 40133 Bologna, Italy.

Ageing, alone or with other factors such as diabetes, may contribute to atherosclerosis. Metabolic and functional alterations of SMC might be implicated. In particular some control mechanisms of cellular proliferation might be alterated during ageing. We studied some growth characteristics of SMC taken from thoracic aorta segments of young male rats (1 month old), adult rats (12 months old) or adult rats with streptozocin - induced diabetes (12 months old). SMC from young rats showed less proliferative capacity in response to 10% calf serum than those from adult healthy or diabetic rats. Moreover adhesion on plastic of SMC from young rats was lower than that of cells taken from adult healthy and diabetic rats (59%, 78% and 82%, respectively). These differences were more marked between 2nd and 4th subcultivations in vitro whereas they were reduced between 4th and 8th subcultivations. We also measured the growth inhibition of SMC by heparin (100μg/ml of non-fractionated heparin alfa-120, Alfa-Wasserman) using both ^{3}H - thymidine incorporation and cell counting. SMC from young rats were inhibited (by about 73% at 4th day of treatment) while the cells from adult healthy and diabetic rats were not inhibited. These data show that the SMC from adult rats with or without diabetes, at early subcultivations in vitro, have a higher proliferative response to stimulation by growth factors. This might contribute to a better understanding of the growth of atherosclerotic plaques during the ageing process.

CG 413 NEURONAL GENE EXPRESSION IS MAINTAINED IN THE ANTERIOR HORN CELLS OF AMYOTROPHIC LATERAL SCLEROSIS, Irma M. Parhad, Arthur W. Clark, Phu M. Tran and Craig A. Krekoski, Department of Pathology, University of Calgary, Calgary, Alta, Canada.
Amyotrophic lateral sclerosis (ALS) is a disorder in which there is a progressive loss of anterior horn cells (ahcs). Studies in ALS have shown a decrement in RNA in surviving ahcs (Davidson & Hartman, 1981); and accumulation of neurofilaments (Nfs) in the proximal axons of these cells (Carpenter, 1968). In order to characterize the role of Nf and other neuronal components in the pathogenesis of this disorder, we evaluated neuronal and glial mRNA levels in 7 pathologically documented ALS and 9 closely matched control cases. Northern, in situ and RNase protection hybridizations were done using human neurofilament light subunit DNA and RNA probes. In addition, Northern analysis was done using the following cDNAs: neuron specific enolase, GAP-43, amyloid precursor protein, and glial fibrillary acidic protein (GFAP). Neuronal counts of ahcs from one lumbar section showed approximately 50% decrease in the ALS cases. Our results showed a slight increase in the mRNA levels of all neuronal markers, but not GFAP, in the ALS cases. This increase did not reach statistical significance. These results indicate that there is no reduction in neuronal mRNA levels in the ALS cases. We conclude: **1)** Despite the prevalence of axonal damage in ALS, surviving ahcs do not show changes in Nf gene expression similar to those seen with axotomy. **2)** Despite disturbance in Nf distribution within the axons, there is no evidence that the axonal changes result from or lead to a significant change in Nf gene expression. **3)** Our current findings do not support the hypothesis that a defect in gene transcription is a primary event in ALS.

CG 414 IN VITRO STUDIES OF HUMAN T LYMPHOCYTE SENESCENCE. Nancy L. Perillo, Roy L. Walford and Rita B. Effros, Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024. We have previously demonstrated a finite, reproducible proliferative lifespan for normal human T lymphocytes cultured in the continuous presence of interleukin-2(IL2). In the more than 100 individual cultures followed, the total number of population doublings (23 ± 7) was precisely within the range found by Hayflick (1965) for adult human lung fibroblasts, allowing the use of human T lymphocytes as an additional model for studying in vitro senescence. During the course of our studies, one putatively "immortal" culture developed from a single stimulation in which all other replicate cultures underwent senescence. By flow cytometry this culture was 100% $CD3^+$ (pan T), 100% $CD4^+$ (T helper) and 100% $CD20^-$ (panB). Karyotypic analysis revealed an additional chromosome 2 in all 20 spreads examined. In addition, the long-lived culture, although IL2-dependent, was found to be less sensitive to IL2 deprivation than the normal cultures. This lends additional support for the claim that the ability to proliferate indefinitely is not a characteristic of normal human T lymphocytes. We are now comparing the long-lived heteroploid cells with normal cells derived from the same donor for the ability to repair benzo(a)pyrenediol epoxide-DNA adducts as well as the extent of DNA fragmentation after IL2 deprivation. (Supported by USPHS AG05309, KO4AG00427 and AG00121).

CG 415 ENVIRONMENTAL LIGHTING AND NUTRIENT DEFICIENCY AS FACTORS IN RETINAL AGING, Laurence M. Rapp and Lisa A. Thum, Department of Ophthalmology, Baylor College of Medicine, Houston, TX 77030
Photoreceptor cell loss is a consequence of retinal senescence in both experimental animals and man. Environmental factors which influence cell loss include chronic light stress and dietary manipulations causing retinal taurine deficiency. Experiments were conducted on albino rats to evaluate the combined influence of light stress and taurine deficiency in accelerating age-related photoreceptor cell loss. Light stress in this study involved maintaining animals in relatively high (300 lux) versus low (2 lux) cyclic illuminance from birth. Beginning at weaning half the animals in both light environments were placed on dietary regimes to deplete retinal taurine. At 3 and 13 weeks of age, measurements of outer nuclear layer area was performed to estimate the total number of photoreceptor cells in the retina. A three-way analysis of variance was applied to the data to examine the influence of age, light environment and taurine deficiency on photoreceptor cell loss. Age-related photoreceptor cell loss did not occur in the low light environment in either taurine-deficient or non-deficient control animals. Controls kept in the high light environment had a 21% loss of photoreceptor cells between 3 and 13 weeks of age. In contrast, animals that were kept in high light environment and made taurine deficient had considerable (65%) loss of photoreceptor cells. These findings indicate that light stress and taurine deficiency act synergistically in causing age-related photoreceptor cell loss.

CG 416 DNA SYNTHESIS INHIBITORS AND THEIR POSSIBLE ROLES IN THE SENESCENCE PHENOTYPE, A. L. Spiering and J. R. Smith, Department of Virology and Epidemiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. We have previously reported the presence of DNA synthesis inhibitors by senescent and quiescent human diploid fibroblasts (HDF). Additionally, we have reported that the immortal human cell line SUSM-1 constituitively produces a DNA synthesis inhibitor. These three inhibitors appear to have different activities based on preliminary characterization studies involving such treatments as temperature, trypsin, cycloheximide and puromycin. Time course studies show further differences between the inhibitors from normal HDF and the immortal SUSM-1 cell line. We feel these inhibitors belong to a new family of negative growth regulators which are turned on in the quiescent state in response to decreased mitogens and in the senescent state in response to a genetic program governing lifespan. SUSM-1 cells do not exhibit either quiescence or senescence. The constitutive expression of the SUSM-1 inhibitor may be the result of a change in this cell line which allows it to escape normal growth control mechanisms present in HDF, resulting in immortality. The ability of an immortal cell line to produce an inhibitor of DNA synthesis, yet be insensitive to the inhibitor lends validity to the hypothesis that immortal cells have achieved immortality through recessive changes in the genetic program leading to senescence.

CG 417 ALTERATION OF DNA REPAIR IN NEURODEGENERATIVE DISEASES OF AGING, J.J. Steinberg, Rod Passman, Angela Scicutella, Janet Gleeson and Peter Davies, Departments of Pathology and Neurosciences, Albert Einstein College of Medicine, Bronx, NY 10461

Neuronal loss remains a hallmark of the neurodegeneration that occurs in senile dementia of the Alzheimer's type (SDAT) and Down's syndrome (DS). Increased susceptibility to ongoing DNA damage, and/or deficiencies in DNA repair may contribute to neuronal demise in SDAT and DS. A model system has been developed to assay the DNA repair enzyme, uracil glycosylase, which is important in maintaining DNA integrity and cell survival. Further, we have increased normal repair mechanism via DNA intercalating agents. We have assayed uracil excision repair in age matched normal, SDAT, and DS brains, and normal and DS fibroblasts. DS excision repair was diminished during gestation, but increased through four years of age, then declined rapidly compared to normal. Aged DS brain retains 12% of normal glycosylase activity (0.13% excision vs. 0.91% excision in SDAT vs. 1.08% excision/ug protein in normal). Further, we have begun to immunologically assay uracil glycosylase with monoclonal antibodies to the enzyme to confirm activities. The intercalating agents (Ames' frameshift test negative) increased repair two-fold over normal fibroblasts. This data supports the idea that diminished DNA repair may be important in degenerative changes evident in SDAT and DS. The consequence of altered repair may be mechanistically involved in accelerated neuronal death or decline. Supported in part by NIH-BRSG #RR-05397.

CG 418 QUANTITATIVE ANALYSIS OF AMYLOID PEPTIDE PRECURSOR RNAs IN ALZHEIMER'S DISEASE, Michael P. Vitek, J. Steven Jacobsen, Robert J. Donnelly, Bernard Beer and Arthur J. Blume, Molecular Neurobiology, CNS Department, Lederle Laboratories, Pearl River, NY 10965

We cloned three different cDNAs encoding amyloid peptide precursors (APPs) of 695, 751 and 770 amino acids (aa) each of which contains the 42aa sequence of the beta-amyloid peptide (BAP) associated with amyloid deposits in Alzheimer disease (AD). Northern blots show that these three APP RNAs, as well as additional APP RNAs, exist in normal and AD brains. Though the qualitative pattern of APP RNA expression may be the same, the amounts of each APP RNA species may be different in normal and AD brain. Using an S1 nuclease protection assay, we have measured the levels of APP 695, APP 751 and APP 770 RNAs in various brain samples. In addition to calculating the APP RNA ratios within a single sample, we have used internal controls to compare APP RNA levels from different samples. From a limited number of brain samples, we observe three distinct patterns of APP RNA accumulation. These results suggest that the post-transcriptional mechanisms regulating the accumulation of the 695, 751, 770 forms of APP RNA may vary between normal and AD brains.

CG 419 ANALYSIS OF THE HETEROGENEITY OF GENERATIONS AMONG CELLS IN SENESCING DIPLOID FIBROBLAST POPULATIONS.
Jeanne Y. Wei and David R. Rigney, Department of Medicine (Cardiology and Gerontology), Harvard Medical School and Beth Israel Hospital, Boston MA 02215. The cells in a cultured diploid fibroblast population have heterogeneous generation times — even cells derived from the same mitosis may divide at different ages. This heterogeneity of inter-mitotic times results in asynchronous growth and a dispersion of generations among members of the cell population. Because the appearance of non-dividing cells in middle age populations has been attributed to the presence of lineages with more generations than average, we estimated the *dispersion* of cell generations as functions of the population doubling level and of the coefficient of variation of intermitotic times. For some data, such as that of Macieira-Coelho and Azzarone [Exp Cell Res 141:325], the rate at which such non-proliferating lineages appear could be explained by a reasonable coefficient of variation of inter-mitotic times (25 percent). Most other data, however, would be fit only if the coefficient of variation of inter-mitotic times were 50 percent or greater, a variability that exceeds what has been observed in microcinematography experiments.

CG 420 GENE EXPRESSION DURING *IN VITRO* CELLULAR SENESCENCE, *Cheryl Wistrom and Bryant Villeponteau, Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109.*
Primary cultured cells normally have a limited life span *in vitro* and mimic various aspects of the aging process seen in intact animals. We have used the diploid cell line MRC-5 to study gene expression during *in vitro* cellular senescence. To identify markers of senescence, we first prepared a cDNA library containing all the genes expressed in senescent cells and then screened the library with labeled cDNA prepared from quiescent young and old cells. From the screening of 4,000 plaques, we obtained 6 senescence-associated clones which all cross-hybridized. The largest of these clones (1.8 kb in length) was named SAG1 which stands for *S*enescence-*A*ssociated *G*ene. Careful Northern blot analysis has demonstrated that SAG1 is a 2.8 kb gene which is expressed some 5-fold higher in old late-passage cells. Control experiments have shown that SAG1 expression does not change during the cell cycle so that the observed differential expression with age cannot be explained by differing cell cycle positions of the cells in early or late passage states. We have partially sequenced SAG1 and checked the BioNet Sequence Data Bank to see if SAG1 is homologous to any known gene. No homologies were found, indicating that SAG1 has not been previously characterized. In other experiments, SAG1 was found to be expressed in SV40 transformed WI38 cells but not in Hela cells. Moreover, SAG1 does not cross-hybridize to rat or chicken DNAs, indicating that SAG1 is very human specific. While SAG1 is the only gene we have identified that is up-regulated in senescent cells, we have identified several genes which are down-regulated with age. Argininosuccinate synthetase, a urea cycle enzyme, is expressed some 10-fold less in old cells and the structural protein collagen is down-regulated some 5-fold.

CG 421 DECREASED KINASE C AND PHOSPHORYLATION OF 87KD PHOSPHOPROTEIN IN SENESCENT HUMAN DIPLOID FIBROBLASTS, Henry C. Yang and Hironori Shigeoka, Department of Medicine, Harbor-UCLA Medical Center, Torrance, CA. 90509
Protein kinase C is a known mediator for growth factor action. Increased in-vitro kinase C activity and kinase C dependent phosphorylation of an 87kD protein (pp87) on 2D gels occur within 1-3 hours after 10% serum or 200nM phorbol 12-myristate, 13-acetate (PMA) treatment of young (population doubling level, PDL = 14-21) IMR 90 human diploid fibroblasts which have been synchronized by low serum arrest. Senescent (PDL = 41-50) cells had a 2-fold decrease of kinase C activity and a 1½-2 fold decrease of pp87 after serum or PMA treatment and during exponential growth. By immunoprecipitation, pp87 appears related to the 87kD phosprotein previosuly described in rat cerebral cortex synaptosomes. Two phosphoproteins of 100kD and 56kD were also visualized and were associated with low serum arrest but not with the senescent state. A phosphoprotein of 95kD was seen and associated with both low serum arrest and senescence but was not kinase C dependent. The data suggest that senescence is associated with a block early in G_0/S and that kinase C and phosphorylation of the 87kD protein may play a regulatory role in senescence.