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Programmed Cell Death in Immune System

O 001 CORTICOSTEROID INDUCED APOPTOSIS IN THE IMMUNE SYSTEM: NOVEL MECHANISMS OF REGULATION

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Apoptosis is a form of programmed cell death that occurs under numerous developmental, physiological and pathological conditions which mandate the selective elimination of cells from tissues or organs. The focus of our research has been to decipher the biochemical mechanisms which mediate glucocorticoid induced apoptosis in the immune system. Chromation degradation is an integral characteristic of all apoptotic cells and thus we have sought to identify, clone and study the mechanisms of regulation of the nucleases which mediate this process. For this purpose we have developed several assays to identify endonuclease(s) whose activity is associated with the onset of apoptosis. Using these assays, we have identified a low molecular weight calcium dependent nuclease whose activity is regulated by glucocorticoids and other activators of apoptosis. The activity of the nuclease is susceptible to inhibition by both zinc and aurintricarboxylic acid which are known to inhibit apoptosis in many cell types. The low molecular weight (18 - 22 Kd) nuclease has been purified to homogenei, microsequenced and cDNA clones used to express the protein in E. coli. Recombinant nuclease is biochemically and pharmacologically indistinguishable from the native rat thymocyte nuclease. Studies on the regulation of the nuclease protein and its gene indicate that this enzyme is present in nuclei of several cell types in a repressed or inactive state. The initiation of apoptotic DNA degradation by glucocorticoids appears to involve enzyme activation and not nuclease induction. Studies toward elucidating the mechanism of nuclease activation are currently underway.

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O 002 GENETIC REGULATION OF PROGRAMMED CELL DEATH. J. John Cohen, Margaret K. Taylor and Cynthia A. Bingham. University of Colorado Medical School, Denver CO 80262.

The term *apoptosis* has come to be used as a synonym for *programmed cell death* by many investigators. Neither term is precisely defined, and the latter is often a misnomer. Apoptosis is a morphologist's term of art; it refers to several peculiar changes seen in some examples of cell death, and it stands in contrast to necrosis. An apoptotic cell shrinks, its plasma membrane becomes ruffled and blebbed in a process called *zeiosis*, its nuclear chromatin collapses into crescents around the nuclear envelope and ultimately into very dense spheres, and the cell is quickly phagocytosed by a healthy neighbor. This final step happens before the cell lyses. The hasty disposal of the apoptotic cell is thought to avoid spillage of its intracellular contents, which would elicit an undesirable inflammatory response. The apoptotic cell displays several surface markers which prompt its phagocytosis.

It is commonly believed that apoptosis occurs in mammals when cell death is "programmed", while necrosis is seen when death is "accidental". However, the concept of programmed cell death is best applied to invertebrates, for which the term was coined, as best illustrated in *Caenorhabditis elegans*. In these animals cell death can be predicted with perfect accuracy, and the genetic regulation of the process is being elucidated. Whether the process of programmed cell death in invertebrates is homologous or analogous to apoptosis is not yet clear.

Apoptosis in mammals has been most extensively studied in rodent thymocytes exposed to glucocorticoids (1). There is evidence that this is an authentic example of physiological cell death, by which cells that are neither positively nor negatively selected in the thymus are removed. It was first shown in this system that apoptosis does not occur if the synthesis of new mRNAs and proteins is blocked by the addition of agents such as actinomycin D or cycloheximide. Numerous other examples of apoptosis also depend upon new macromolecular synthesis; we call these *induction* mechanisms. However, there are instances of apparent apoptosis in which neither mRNA nor protein synthesis are necessary; they may be called *transduction* mechanisms, because the main example is the transduction of apoptosis in tumor cells by cytotoxic T cells. The existence of such models suggests that all the molecules necessary for apoptosis are already present in most cells; the apparently paradoxical relationship to induction has not yet been resolved. There are also *release* mechanisms, in which apoptosis occurs upon the inhibition of macromolecular synthesis. Linking these disparate systems in a common model will be an interesting challenge.

We have begun a genetic approach to apoptosis by isolating mRNAs expressed in thymocytes after exposure to dexamethasone (2). Death-associated messages were enriched by subtractive hybridization, followed by differential screening of control and "programmed" cDNA libraries. Candidate inserts were used to probe Northern blots of RNA from unrelated cells undergoing apoptosis, to minimize the isolation of thymus-specific or glucocorticoid-specific messages. Several death-associated messages have been isolated. One, RP-8, is probably a transcriptional regulatory factor. Its expression is increased in apoptotic cells in a variety of tissues including thymus and brain. The limitations and advantages of the genetic approach must be considered in the light of what we know about the triggering of apoptosis.

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O 003 OOCYTE LOSS CAUSED BY A DISRUPTION OF IMMUNE SYSTEM MATURATION, Sandra D. Michael and John C. Chapman, Binghamton University, State University of New York, Binghamton, New York 13902-6000.

Experimental evidence has made it clear that hormones play an important role in regulating the immune response. Recent studies have also shown that alterations in the immune system produce significant effects on the endocrine system, suggesting that the relationship of the thymus with these other endocrine glands is dependent upon a series of interactive functions, or axes of communication. Here we will elaborate on the reproductive axis: the hypothalamus-pituitary-gonadal-thymic (HPGT) network and the effects of its disruption on accelerated oocyte loss. Experimental studies of the effects of the endocrine thymus on the gonads have primarily used genetically athymic or neonatally thymectomized animals. The absence of a thymus gland, or its removal in both male and female mice and rats, alters gonadal development and function, affects levels of circulating pituitary and gonadal hormones, and disrupts the maturation or function of T cells. The available evidence suggests that thymus-gonadal interactions are mediated by two interrelated mechanisms. The first depends upon the normal function and relative proportions of various T cells, and the second depends upon hormonal interactions among the endocrine glands. The first mechanism can be illustrated with a brief summary of the theory underlying the development of autoimmune oophoritis in the neonatally thymectomized female mouse. In intact females, the presence of ovarian antigens is tolerated because of the existence of ovarian tissue-specific suppressor T cells. This particular subset of thymic-derived lymphocytes inhibits ovarian tissue-specific helper T and/or amplifier T cells. In Tx-3 mice (thymectomized on day 3 of age), helper T and/or amplifier T cells have matured, or are capable of maturing, prior to the day of thymectomy. In the Tx-3 mouse, the suppressor T cells are absent or nonfunctional because they apparently require longer than 3 days after birth to develop. In the absence of the suppressor T cells, helper T cells assist the B cells to produce autoantibodies against component(s) in the ooplasma of the oocytes. The resultant ovarian dysgenesis is characterized by lymphocyte infiltration, a rapid loss of oocytes, and a subsequent decrease in the number of follicles. Thus, removing the thymus gland before the HPGT axis is fully developed disrupts thymic modulation of normal effector lymphocyte development, thereby causing rapid senescence of the follicular apparatus. During its accelerated senescence the apparatus alters the levels of estradiol, reduces the levels of progesterone, and elevates the levels of testosterone. Within intact animals, ovarian steroids via feedback mechanisms alter pituitary hormones, and these in turn modulate the thymic hormones. The loss of thymic hormones through thymectomy may also be a contributing factor to the altered steroidogenesis by a direct action of thymic hormones on the hypothalamus-pituitary-gonadal endocrine pathway. Thus, the interaction of the thymus gland with the ovary may be directly mediated through effector T cells, the first mechanism, and indirectly through the traditional reproductive hormone pathway, the second mechanism.

Cell Death: Models from Development and Senescence

O 004 EARLY METABOLIC EVENTS AND UP-REGULATED GENES DURING PROGRAMMED CELL DEATH (PCD), Richard A. Lockshin[1], Reginald Halaby[2], Theresa E. Latham[1,2], and Zahra F. Zakeri[2], [1]Dept. of Biol. Sci., St. John's Univ., Jamaica NY 11439 and [2]Dept. of Biology and Graduate Center of CUNY, Queens College, Flushing, NY 11367.

Programmed Cell Death (PCD) appears to require RNA and protein synthesis but the function of the newly-synthesized proteins is not known. Numerous gene products have been shown to be up-regulated, but up-regulation may occur in response to metabolic changes, as a non-controlling aspect of PCD, or as a result of loss of repressors. We are attempting to analyze this problem by comparing mRNA and protein synthesis in two different dying tissues. In metamorphosing *Manduca sexta*, (Lepidoptera) the pattern of incorporation of ^{35}S methionine into proteins in both muscle and labial gland alters as involution begins and finally reduces to a small number of proteins synthesized during late degeneration. To study this response, we are isolating cDNAs from up-regulated genes common to the two tissues and expressed during the latter stages of cell death. We have constructed cDNA libraries from various times for differential hybridization. A day 3 gland cDNA library in λgt11 vector was probed with PCR amplified λ gt10 muscle 12-18 hr cDNA library. We screened 10^5 clones and isolated 9. Of these, 6 were positive after secondary and tertiary screenings. Subsequently, these clones from late degenerating gland were probed with PCR amplified 0-6 hr and 6-12 hr muscle cDNA libraries. The signal is most intense with the probe from 12-18 hr muscle and vanishingly small against a probe from 0-6 hr muscle. We are quantifying message level by slot blot hybridization against PCR-generated putative clones. Since these clones appear to be common to two very different dying cells, we suggest that they may play a role in programmed cell death.

We are also examining early metabolic changes in these tissues. In both insect and embryonic mouse, fragmentation of DNA is a late and relatively minor event, suggesting that the prominence of DNA laddering depends on the tissue and species. In *Manduca* labial gland, many metabolic changes precede DNA laddering. Among the earliest that we detect are a modest rise in cyclic GMP, an increase in lysosomal enzymes, and a decrease in overall protein synthesis. The latter phenomenon has also been noted by others in de-afferented neurons and in cultured CNS-derived cells deprived of NGF. Early metabolism of PCD is a neglected field that should be studied more intensely. In order to understand the role of PCD and apoptosis in aging, and perhaps to regulate it, we must resolve these several questions.

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Neurotoxin-Aging Interactions

O 005 MOLECULAR MECHANISMS OF ALZHEIMER'S DISEASE NEURODEGENERATION, Rachael L. Neve[1], Michael R. Kozlowski[2], Joshua A. Donis[1], Montserrat Ventosa-Michelman[1], Stephen Bossi[1], Anja Kammesheidt[3], and Christine F. Hohmann[4], [1]Molecular Neurogenetics Laboratory, McLean Hospital, Harvard Medical School, Belmont, MA 02178, [2]Bristol-Myers Squibb Company, Pharmaceutical Research and Development Division, Wallingford, CT 06492, [3]Department of Psychobiology, University of California, Irvine, CA 92717, [4]The Kennedy Institute, Baltimore, MD 21205.

The neuropathology of Alzheimer's disease is characterized both by the deposition of amyloid in senile plaques and along the walls of the cerebral blood vessels and also by the degeneration of neurons, which is accompanied by the intracellular formation of neurofibrillary tangles. The 39-43 amino acid fragment termed beta/A4 is the primary component of the amyloid deposits. The isolation of cDNAs containing the beta/A4 coding sequence revealed that the beta/A4 found in amyloid deposits in Alzheimer's disease represents a peptide derived from a larger precursor protein.

The mechanism of beta/A4 production in Alzheimer's disease remains unclear. Although the normal processing of the amyloid precursor protein involves cleavage of the protein within the beta/A4 sequence, a minor lysosomal processing pathway in the brain appears to yield several potentially amyloidogenic beta/A4-containing carboxyterminal fragments. Thus, the pathological accumulation of the beta/A4 polypeptide in Alzheimer's disease brain does not necessarily result from an abnormal cleavage event, but may instead accrue from a cellular shift to the lysosomal processing pathway for the amyloid precursor protein. We have shown that the carboxyterminal 100 amino acids of the amyloid protein precursor (APP-C100) is neurotoxic. The neurotoxicity of this carboxyterminal APP fragment suggests that it might play a role not only in amyloidogenesis but also in the development of the progressive neuropathology of Alzheimer's disease.

We recently demonstrated that APP-C100 causes the degeneration of neurons by interacting with a neuronal cell surface receptor. We have hypothesized that APP-C100 is a devastatingly neurotoxic intermediate generated during the formation of beta/A4 from the amyloid protein precursor and that this peptide, rather than beta/A4 per se, causes neuronal pathology and death in Alzheimer's disease. To test this hypothesis, we made two different animal models in which this fragment of the amyloid precursor protein was expressed in the brain. In the first model, we transplanted genetically modified cells expressing APP-C100 into newborn mouse brains. Ths mice developed severe cortical atrophy and displayed markers of Alzheimer's disease pathology by four months of age. In the second model, we made transgenic mice expressing APP-C100 in the brain. These mice also display pathological features that are characteristic of Alzheimer's disease.

O 006 B-AMYLOID PEPTIDE (B-AP) INDUCED COMPLEMENT ATTACK IN ALZHEIMER'S DISEASE, Joseph Rogers, Sun Health Research Institute, Sun City, AZ 85372.

Numerous markers of immune function have been observed in the Alzheimer's disease (AD) brain. These include activated (HLA-DR$^+$) scavenger cells (microglia), cytokines, cytokine receptors, classical pathway complement proteins, complement receptors, and complement regulatory proteins. Most of these immune response elements are uniquely elevated in the AD association and limbic cortices, and co-localize with compacted B-AP deposits.

Complement activation in the AD brain could be a significant pathogenic event. The anaphylatoxins C3a and C5a serve as chemotactic stimuli that direct scavenger cell attack. These proteins are found in the AD brain, as are microglia with anaphylatoxin receptors. The membrane attack complex, C5b-9, is a second complement cytopathic mechanism that has been demonstrated in the context of compacted B-AP deposits. Since these deposits are known to coincide with regions of neuronal and neuritic damage, complement mediated cytopathology may be one of the underlying pathogenic factors.

Recent experiments provide further detail on complement cytopathology in AD, and may help bridge the gap between this body of research and that on B-AP. In vitro, B-AP binds C1q, the first component of complement, in an antibody independent fashion, fully activating the classical pathway. Though novel, this phenomenon is not unprecedented, occurring with several other peptides (e.g., mannose binding protein) and in human diseases such as congenital C1 inhibitor deficiency.

It is not uncommon for the extent of B-AP deposition in a clinically nondemented patient to be as great as that observed in an AD patient. This frequently ignored but easily verified finding suggests that B-AP may be a necessary but not sufficient pathogenic element, requiring other factors such as complement to achieve neurotoxicity. Consistent with this idea, full complement activation co-localized with B-AP is present in virtually all AD patients, and absent in nondemented patients.

The ultimate test of whether this research has therapeutic significance will rest on clinical trials. To date, both retrospective studies and a Phase 1 trial of Indocin show promising results.

Clonal Senescence-I

O 007 INHIBITORS OF DNA SYNTHESIS DERIVED FROM SENESCENT HUMAN DIPLOID FIBROBLASTS, James R. Smith, Baylor College of Medicine, Houston, TX 77030.

Previous work from cell fusion experiments suggested that cellular aging was a genetically programmed process. These experiments involved the fusion of young normal fibroblasts to senescent fibroblasts, normal fibroblast-like cells with various immortal cell lines and the fusion of various immortal cell lines among themselves. The results of these experiments suggested that cellular aging was the result of the proper functioning of a genetic program. A loss of function of any one of four different processes or pathways could result in cellular immortality. The questions raised by this set of experiments were: what kind of genetic program determined the limited in vitro lifespan of normal cells in culture, what was the endpoint of this genetic program, and how did this lead to the inevitable cessation of division observed in normal human cells in culture. Considerable evidence has accumulated over the past several years indicating that when normal human fibroblasts become senescent, they produce an inhibitor of DNA synthesis. Membranes isolated from senescent cells and added to young cell cultures were capable of inhibiting the initiation of DNA synthesis, suggesting that the inhibitor resided in the membrane fraction of the senescent cells. Subsequently, proteins were extracted from either monolayers of senescent cells or from isolated membrane preparations using a mild non-ionic detergent. These crude protein preparations were also able to inhibit initiation of DNA synthesis when added to young cell cultures. In a further experiment aimed at determining the feasibility of cDNA cloning of the inhibitor of DNA synthesis, we reported that microinjection of the poly A^+ fraction of RNA isolated from senescent cells into young cells blocked initiation of DNA synthesis. The non-poly A fraction, RNAse treated poly A fraction or RNA isolated from young cells were unable to block the initiation of DNA synthesis. Taken together, these experiments provide strong evidence that senescent cells produce a gene product that is able to inhibit initiation of DNA synthesis, as assayed by its effect on young proliferation-competent human fibroblasts. Recently, we have isolated three cDNA clones that inhibit initiation of DNA synthesis in young human fibroblasts, following either transfection into the young cells or microinjection into the nucleus of the young cells. Two of these three code for messenger RNAs which have the same relative abundance in young and senescent cells. One of the clones termed SDI-1 has a messenger RNA which is elevated by 10-20 fold in senescent cells compared to young cells. The level of the mRNA for SDI-1 is also elevated in cells made quiescent by serum deprivation for 2-3 weeks. In this regard, SDI-1 resembles the characteristics of the inhibitor of DNA synthesis that we had sought earlier.

O 008 TUMOR SUPPRESSOR GENES AND THE REGULATION OF CELLULAR SENESCENCE, Woodring E. Wright, Walter D. Funk, Michel Ouellette and Jerry W. Shay, University of Texas Southwestern Medical Center, Dallas, Texas 75235.

SV40 T-antigen extends the lifespan of human diploid lung fibroblasts from approximately 50 population doublings (PDL) to 70 PDL. In rare cases, an immortal focus of cells appears that overgrows the culture and gives rise to an established cell line. By using a T-antigen construct in which expression was controlled by an inducible promoter, we demonstrated that both the extension of lifespan and proliferation following the immortalization event required the continued expression of T-antigen. This led to the hypothesis that there were two independent mechanisms regulating cellular senescence. Mortality Stage 1 (M1) would be induced after approximately 50 doublings, and could be inactivated by T-antigen. In the presence of SV40 T-antigen, the cells would then divide until the Mortality Stage 2 mechanism was induced, causing crisis. The rare cell that spontaneously inactivated the M2 mechanism, probably by mutation, would then give rise to the immortal cell line. We later showed that two tumor suppressor genes, the retinoblastoma gene product (RB) and p53, were the primary regulators of the M1 mechanism.

Work from other laboratories has focused attention on the mechanisms by which cells could "count" the number of times they had divided. The discovery that normal cells lack the enzyme (telomerase) that maintains the ends of chromosomes, so that telomeres shorten by about 50 bp per cell division in normal fibroblasts, provides the key concept. The current integration of the telomere shortening hypothesis and the two-stage mechanism for cellular aging is based on the observation that telomeres are usually heterochromatic, and that heterochromatic regions can effect the expression of genes trapped within them. We postulate that the regulatory factors controlling the M1 mechanism are located in sub-telomeric DNA, and that the length of the telomere effects the heterochromatic state of this subtelomeric region. After approximately 50 doublings, when the length of the remaining telomere repeats is about 2 kbp, the heterochromatic state of the subtelomeric DNA changes so that the M1 program is induced. RB and p53 are then modified to be in a constitutively active state. In the presence of T-antigen and the inactivation of the M1 mechanism, the cells continue to divide. After 20 additional doublings, the remaining telomeres are sufficiently short that many chromosomes would lack telomeric repeats, and the ensuing consequences would cause crisis. Escape from crisis would occur by a mechanism that involved the reinduction of telomerase activity. A convenient model for this is to postulate a mutation in one of the subunits of a complex involved in repressing telomerase activity. The combination of inactivating M1 and regaining telomerase activity would then result in an immortal cell line.

Clonal Senescence-II

O 009 FUNCTIONAL AND MOLECULAR GENETIC ANALYSIS OF *IN VITRO* SENESCENCE IN HUMAN T LYMPHOCYTES, Rita B. Effros, Nancy L. Perillo, Faramarz Naeim, Xiaoming Zhu and Roy L. Walford, Department of Pathology and Laboratory Medicine, UCLA School of Medicine, Los Angeles, CA 90024.

Human T lymphocytes display a limited *in vitro* lifespan, as is the case for all normal diploid mammalian cells. Long term cultures of normal peripheral blood T cells, activated *in vitro* and passaged in the presence of interleukin 2 undergo 23 ± 7 cumulative population doublings, a figure which is nearly identical to that obtained for adult fibroblasts. The concordance in life span of two vastly different cell types, one grown in monolayer in the presence of serum, and the other grown in serum-free suspension culture, reinforces the biological significance and the probable genetic basis for *in vitro* senescence. Senescent T cell cultures will not proliferate, even if restimulated with the original allogeneic stimulator cells. However, such restimulation induces an increase in the percentage of cells expressing the p55 chain of the IL2 receptor, and this upregulation is comparable to that observed in a "young" culture which underwent two population doublings during the same time period after restimulation. The senescent T cells, primarily of the $CD8^+$ phenotype, are highly potent and specific cytotoxic effector cells in a chromium release assay. In addition, the surface expression of LFA-1, CD2, CD44 (Pgp-1), CD29, HLA-DR, and UCHL-1 antigens, all characteristic of *in vivo* derived memory T cells, persists throughout the lifespan of the culture. Although no change in cell size was observed between early and late passage cells (a finding which differs from the fibroblast system), cell cycle analysis revealed that antigenic restimulation resulted in only 16-22% of the senescent cells entering S phase, in contrast to 60% in the young cultures. A second significant characteristic of the senescent T cells is the diminished production of hsp70 following a 42° heat shock. Electrophoretic mobility shift analysis suggests that the reduction is at the level of transcriptional control. The progressive decline in the stress response relates to the culture age rather than the actual proliferative activity: comparison of recently restimulated and non-restimulated pre-senescent cultures showed identical heat shock response indices, despite vastly different degrees of proliferation. Additional experiments on other T lymphoid cells confirmed this conclusion: cultures from a rapidly proliferative T cell tumor and an immortal T cell variant show a reduction in HSP70 similar to that of senescent normal T cells. By contrast, normal unstimulated peripheral blood T lymphocytes, which are not proliferating, produce extremely high levels of hsp70. We conclude that for senescent T cells the reduced ability to respond to heat shock by producing HSP70, although lying at the level of transcriptional control, may nevertheless be unrelated to the reduced DNA synthesis or reduced proliferative activity also manifested by these cells. Furthermore, our findings are consistent with the hypothesis that senescence is a programmed event rather than a generalized decline in the functional activity of a differentiated cell. (supported by AG05309, AG00121, and AG00427)

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Genomic Stability

O 010 AGE-DEPENDENT DNA ALTERATIONS (I-COMPOUNDS) AS ASSAYED BY ^{32}P-POSTLABELING, Kurt Randerath and Erika Randerath, Division of Toxicology, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030.

Mammalian tissues display species-, strain-, sex-, and tissue-specific DNA adductlike covalent DNA modifications termed I (indigenous)-compounds, whose levels increase with age and are modulated by dietary factors (1-5). I-compounds do not appear to be derived from exogenous (e.g., dietary) carcinogens, thus their formation presumably results from the action of endogenous metabolites arising in the course of normal intermediary nutrient metabolism. I-compounds are readily detected by the nuclease P1-enhanced ^{32}P-postlabeling assay (6) and have been observed in all tissues of unexposed animals investigated thus far (12 different rodent tissues) and in human brain. Chromatographic properties of I-compounds suggest diverse chemical structures, but owing to their low levels in tissue DNA, chemical identification has not yet been accomplished. High carbohydrate and high protein diets enhance rat hepatic and renal I-compound levels compared with high fat diets, and natural ingredient diets produce more complex profiles and higher levels of I-compounds than do purified diets (4). Caloric restriction (by 40%), the most effective means to extend life span and improve resistance to carcinogenesis and a variety of age-related lesions in rodents, was found to consistently elevate I-compound levels in rat tissues up to two-fold (7). Age-dependent increases of many individual I-compounds are linear in caloricaily restricted but not ad libitum fed rats (8). On the other hand, exposure to carcinogens and tumor promoters (e.g., 2,3,7,8-tetrachlorodibenzo-p-dioxin, choline-devoid diet, peroxisome proliferators, carbon tetrachloride, polychlorinated biphenyls, phenobarbital, 3-methylcholanthrene, and 2-acetylaminofluorene) reduces I-compound levels in the target organ (3,9,10). Such reductions, which are dose- and exposure time-dependent, appear to be due to both decreased I-compound formation and increased cell proliferation. Smaller numbers and lesser amounts of I-compounds are also found in target organs of spontaneous carcinogenesis and degenerative diseases and in hepatic tumors (11,12). In contrast to carcinogen-DNA adducts, levels of several I-compounds in mouse liver and rat liver show circadian variation (13), implying precise regulation of I-compound levels in vivo. This effect may conceivably be related to circadian rhythms of DNA functions. The association of enhanced I-compound levels with caloric restriction and of reduced levels with the carcinogenic process and other pathology suggests that at least some I-compounds may not primarily reflect "DNA damage". Our data are consistent with, but do not prove, the hypothesis that despite their carcinogen adductlike character, I-compounds may play a protective role in carcinogenesis and age-related degenerative diseases.

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O 011 MODIFICATION OF PROTEIN BY OZONE, METAL ION-CATALYZED REACTIONS, AND BY LIPID PEROXIDATION PRODUCTS. Earl R. Stadtman, Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

During aging a number of enzymes accumulate as catalytically inactive or less active forms. These age-related changes are due in part to: (a) modification of the side chains of some amino acid residues by metal ion-catalyzed oxidation (MCO) systems, by ozone, and by reactions with oxidation products of polyunsaturated fatty acids; (b) fragmentation of the polypeptide chains; (c) aggregation of polypeptide chains via covalent cross-linking reactions; and (d) a decrease in the ability of intracellular protease to degrade the modified proteins. A number of enzymic and nonenzymic MCO systems catalyze the reduction of Fe(III) to Fe(II) and of O_2 to H_2O_2, which react at metal binding sites on enzymes to produce active oxygen species (HO$^{\cdot}$, ferryl ion), which attack side chains of amino acid residues at the metal binding sites. Among other modifications, carbonyl derivatives of some amino acid residues (i.e., proline, arginine, and lysine residues) are formed; histidine residues are converted to asparagine or 2-oxoimidazolones; proline residues are converted to glutamate, pyroglutamate, or 2-pyrrolidine derivatives; and methionine residues are converted to methionine sulfoxide residues. Except for histidine, the aromatic amino acid residues are not primary targets for modification by MCO systems. However, aromatic residues and cysteine and methionine residues are readily modified by ozone or hydroxyl radicals generated by radiolysis. Both treatments have been shown to convert tryptophan residues to formyl kynurenine and tyrosine and phenylalanine residues to polyhydroxy and carbonyl derivatives. The generation of protein carbonyl derivatives is obtained also by interaction with oxidation products of polyunsaturated fatty acids. One such product, 4-hydroxynonenal, reacts with histidine, lysine, and cysteine residues of proteins by Michael addition mechanisms, to form adducts possessing a carbonyl function. These may undergo secondary Schiff base-type reactions to produce inter- and intra-protein crosslinkages. Such reactions likely contribute to the pool of inactive enzymes that increases in size with age. These age-dependent changes in enzyme structure and activity likely reflect the accumulation of random, free-radical induced alterations of a multiplicity of genes that govern the balance between protein modification on the one hand and the degradation of modified protein on the other.

Genetics of Longevity in Animal Models

O 012 GENETIC AND MOLECULAR ANALYSIS OF LONGEVITY ASSURANCE GENES IN DROSOPHILA, Robert Arking[1], Steven P. Dudas[1], Steven Buck[1], Allen Force[1], Michael Nicholson[1], and George Baker[2], [1] Wayne State University, Detroit and [2]National Institute of Aging, Baltimore.

Aging is a complex process. The identification of the mechanisms controlling the aging process has proven to be a difficult problem. In the absence of any firm knowledge regarding aging mechanisms, we have chosen to adopt a genetic analysis of extended longevity in Drosophila. Our past work has allowed us to demonstrate that **(1)**, artificial selection has increased mean and maximum life span by ca. 40% in the selected strain (L) relative to the contol (R) strain; and **(2)**, the extended longevity phenotype (ELP)is due to a delayed onset of senescence, which takes place between 5-7 days of adult life as judged by biomarker analysis. The events regulating longevity and senescence take place early in life. **(3)**, A complete genetic analysis, identifying the role of each chromosome in the expression of the ELP, has been done. The recessive genes responsible have been localized to the 3rd chromosome (c3). Genes on other chromosomes are involved in the expression of the ELP but are not expressed in the absence of the c3 genes. Isozyme data supports the implied *trans* and regulatory effect of the c3 genes. There are epistatic effects such that the c3 genes may be repressed by genes on c2, which themselves may be repressed by genes on c1. Thus there exists a hierarchy of specific genes which regulate the expression of the ELP and it can only be expressed in specific genotypes. **(4)**, However, the ELP is inducible since, even with the correct genotype, the ELP is only expressed in animals raised under high larval density (HD) conditiions. Raising L strain animals under low larval density (LD) conditions results in the animals' having only a normal life span. The L-HD animals weigh less than do their genetically identical L-LD sibs. The critical period for exposure to HD conditions is 60-100 hrs post oviposition. **(5)**, During this period, there are statistically significant changes in ADH mRNA and enzyme activity in the L-HD strain only. This is followed by a coordinated increase in specific antioxidant mRNA levels and enzyme activities in the 5 day old L-HD adult, a time point which just precedes the delayed onset of senescence. **(6)**, These increases are functional, as shown by the fact that the L-HD animals show an increase in their resistance to exogenous paraquat at this same time. The control (R-HD) strains do not show such elevations and shortly thereafter show a decrease in their paraquat resistance. This is then followed by the loss of certain behavioral biomarkers. **(7)**, In summary, the events that occur during the critical period involve the activation of specific genes, beginning in the 3rd larval instar and culminating in a series of coordinated changes in gene activities which occur in the 5 day old adult. These activities lead to the delayed onset of senescence and the expression of the ELP.

O 013 **MOLECULAR GENETICS OF *C. ELEGANS* LIFE SPAN**, Gordon J. Lithgow, Pat Tedesco, Edward W. Hutchinson, Simon L. Melov, Anne Jensen, Stacy Duhon and Tom E. Johnson, Institute of Behavioral Genetics, University of Colorado, Boulder, Colorado, USA.

Long-lived strains of the nematode *C. elegans* are being utilized in a number of ways to define genes that determine life span and to test mechanistic theories.

A number of genes, which when mutated lead to extended life span, have been defined by mutation. The first of these genes, *age-1*, extends life span by up to 70%. We have positioned this gene on the genetic map by a novel multipoint mapping scheme and onto the physical map by a positional cloning approach. Cloning of the gene is being pursued by a number of molecular techniques. The successful definition of a single gene limiting life span has lead us pursue further screens for long lived mutants. Three additional long lived strains have been isolated and one of these appears to be an allele of *age-1*. The observation that strains carrying the *age-1* mutation exhibit elevated free radical scavenging enzyme activities in late life has prompted an analysis of stress response in various strains. The *age-1* mutation appears to be associated with resistance to the free radical generating agent, paraquat. We have mapped the resistance phenotype to the *age-1* locus and are investigating the association of resistance with genetically defined life span. In addition, we are investigating the expression of other genes which are implicated in stress response in long lived and normal lived strains. The ease with which *C. elegans* can be manipulated genetically may facilitate the testing of mechanistic theories developed in other systems. We view this as a two stage process. Firstly, we establish corollary between a biochemical or molecular property and genetically determined life span. Secondly, we intend to establish causal relationships by the manipulation of candidate gene expression.

O 014 **GENETIC AND PHYSIOLOGICAL MECHANISMS OF POSTPONED AGING IN DROSOPHILA**, Michael R. Rose[1], Robert H. Tyler[1], Greg S. Spicer[2], Francisco J. Ayala[1], Theodore J. Nusbaum[1], Laurence D. Mueller[1], Joseph L. Graves[1], and James E. Fleming[2], [1]University of California, Irvine, [2]Linus Pauling Institute, Palo Alto, California.

Drosophila melanogaster with postponed aging have been created by selection for later reproduction over the last twelve years. These flies now live about 80% longer than their matched controls. Flies with further postponement of aging have been produced by selection for increased resistance to two lethal stresses: starvation and desiccation. The longest-lived flies now live about 100% longer than the original control flies. The longer-lived flies have enhancements in a variety of performance characters: flight duration, resistance to starvation, resistance to desiccation, resistance to ambient ethanol, later locomotion, and later fecundity. Crosses using longer-lived flies suggest that many loci are involved in the response of these stocks to selection. Comparisons of longer-lived flies with their controls using two-dimensional gel electrophoresis suggest that a few percent of all D. melanogaster loci may be involved in the postponement of aging. However, a few percent of 10,000 or so loci is still hundreds of loci. One-dimensional protein gel electrophoresis indicates that Cu,Zn superoxide dismutase, or a locus closely linked to it, is involved in the control of aging in these stocks: longer-lived populations have increased frequencies of the more active SOD allele. Resampling different SOD alleles from nature does not yield detectable differences in lifespan, however. This suggests that the contribution that SOD makes to the postponement of aging in these stocks is relatively small. This result in turn indicates that many loci of individually small effect control aging in Drosophila, illustrating the importance of quantitative genetic techniques in the study of aging.

O 015 **LIFESPAN-DETERMINING GENES MAP TO AT LEAST 7 CHROMOSOMAL LOCI IN *C. ELEGANS***

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We have obtained a coarse mapping of genes which modulate lifespan in the nematode *C. elegans*. Using transposable elements as heteroallelic markers to distinguish between the parental strains' genomes, we identified genotypes across the chromosomes of the longest-lived individuals in a large, heterogeneous recombinant-inbred (RI) population. We first generated complex RI populations with high genetic heterogeneity, from which large synchronous aging cohorts were prepared. We then characterized the distribution of parental alleles at 25 polymorphic markers, using polymerase chain reaction to amplify DNA from single worms. Regions were thus identified at which *either allele* was overrepresented in the longest-lived subpopulation, relative to young controls. We observed changes in allele frequency at 7 loci: one each on chromosomes I – V, and two on the X chromosome ($p<0.01$ – $p<0.05$). The strongest allele selection, 1.8- to 2-fold, affected 4 markers spanning a dense gene cluster on chromosome II, centered near *age-1* (the only gene known to increase lifespan when mutated) and *sod-1*. Similar results were obtained in duplicate mappings, for which long-lived worms were maintained in two very different environments: (a.) worms kept at low density, in Petri dishes, hand-transferred to fresh medium to eliminate progeny; or (b.) in high-density, vigorously-aerated flask cultures, with 100 μM FUdR added at maturity to prevent reproduction. The similarity of outcomes indicates that the resolved genes govern lifespan, rather than adaptation to specific environments. These results provide the first estimate of the number of genes with the potential to alter lifespan, and their initial localization. Because not all such genes are expected to be polymorphic between the two strains utilized, the number of loci implicated in these experiments is only a lower limit.

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Post-Translational Errors in Aging

O 016 SPONTANEOUS DEGRADATION OF AGING PROTEINS: CAN ENZYMES REVERSE THE DAMAGE? Steven Clarke, Department of Chemistry and Biochemistry and the Molecular Biology Institute, UCLA, Los Angeles, CA 90024-1569.

Precise structures allow proteins to carry out their architectural, catalytic, and signalling roles. However, spontaneous reactions that occur under physiological conditions, including oxidation, glycation, isomerization, racemization, and deamidation, lead to alterations of structure that can compromise protein function. Asparaginyl and aspartyl residues represent two of the major sites of damage, where intramolecular reactions result in the accumulation of isoaspartyl and D-aspartyl derivatives.

The accumulation of inactive proteins containing altered aspartyl residues may cause particular damage to aging cells, especially those where individual cells must function for extended periods (neurons, spermatozoa, oocytes), or when protein synthesis is limited (red blood cells), or when both conditions prevail (eye lens). The discovery of an enzyme that specifically recognizes proteins with altered aspartyl residues in a variety of cell types has suggested the possibility that such modified proteins could be metabolized. This enzyme catalyzes the S-adenosylmethionine-dependent methyl esterification of L-isoaspartyl and D-aspartyl residues. Studies with peptide model systems show that the methylation reaction can be the first step in a repair pathway that can relatively efficiently convert altered L-isoaspartyl residues to normal L-aspartyl residues. These results suggest that this methyltransferase may function in a protein repair system to limit the damage caused by the accumulation of aged proteins.

To directly study the physiological role of this methyltransferase, we examined the ability of the bacterium *Escherichia coli* to survive in in the absence of its activity (1). This enzyme is one of the most highly conserved proteins in nature (31% of the residues of the bacterial and human enzymes are identical) suggesting that the bacterial system may be a useful model for understanding protein repair in mammalian cells. We utilized gene disruption techniques to produce mutants that have no detectable methyltransferase activity. Although no changes in exponential-phase growth were observed, the mutants did not survive well upon extended culture into stationary phase or upon heat challenge, both conditions where the accumulation of altered proteins would be expected to limit cell function. These results provide genetic evidence for the role of this methyltransferase in the metabolism of altered proteins that can accumulate in aging cells and limit their viability.

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O 017 MUTATIONS OF MITOCHONDRIAL DNA: MOLECULAR BASIS OF AGING AND HUMAN DEATH?, Bernhard Kadenbach[1], Christof Münscher[1], Theophil Rieger[1] and Josef Müller-Höcker[2], [1]Fachbereich Chemie, Philipps-Universität Marburg, and [2]Pathologisches Institut der Universität München, Germany.

The energy of mammalian cells is mainly synthesized in mitochondria by oxidative phosphorylation (about 95 % of total cellular ATP). The maternally inherited mitochondrial DNA (mtDNA) codes for only 13 proteins, which are exclusively subunits of proton pumps, i.e. complexes I, III, IV and V of the mitochondrial respiratory chain. During the last few years various forms of human mitochondrial encephalomyopathies could be related to point mutations or deletions of mtDNA. The maternal inherited MERRF disease (Myoclonic Epilepsy with Ragged Red Fibers) could be associated with an A to G transition at position 8344 within the mitochondrial tRNALys gene. The MERRF-mutation was shown to decrease mitochondrial protein synthesis (1). The "common deletion" was found in mitochondrial diseases without proven maternal inheritance like Kearns Sayre Syndrome, which are histochemically characterized by a focal (cell- or fiber-specific) defect of cytochrome c oxidase (complex IV).

An increasing number of cytochrome c oxidase defective cells could be identified histochemically mainly in the heart of healthy humans with increasing age (2). In addition deletions of mtDNA were shown to accumulate in human brain and skeletal muscle during normal aging. By applying a point mutation specific polymerase chain reaction (PS-PCR) we could also identify very recently the MERRF point mutation in skeletal muscle of elderly people together with the "common deletion" (3). None of these mutations of mtDNA could be found in navel-string samples of 5 newborns and in HeLa cells. The data are in agreement with the previous proposition that statistic somatic mutations of mtDNA may represent the molecular basis of aging and the final human death (4).

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O 018 PROBING OF THE AGING PROCESS WITH ADVANCED MAILLARD REACTION PRODUCTS, Vincent M.Monnier[1], David A. Sell[1], R.H. Nagaraj[1], John Fogarty[1], Case Western Reserve University, Institute of Pathology, Cleveland, OH 44106

Proteins that are incubated with reducing sugars under physiological conditions undergo age-related modifications reminiscent of changes occurring in long-lived proteins *in vivo*, such as yellowing and cross-linking. Pentosidine is a fluorescent lysyl-arginine crosslink which was originally isolated from human extracellular matrix. Pentosidine formation increases with age linearly in dura mater, exponentially in skin and follows an asymptotic plateau-shaped pattern in purified glomerular basement membranes. Skin pentosidine levels are elevated in diabetes and were found to correlate with severity of complications suggesting an association between cumulative level of glycemia and tissue damage. On the contrary, tail tendon pentosidine was decreased in dietary restricted Fischer 344 rats. Dramatic elevations in plasma levels were found in uremia. They were normalized upon renal transplantation, suggesting accumulation of pentosidine precursors by the diseased kidney. These observations and the fact that several features of aging such as atherosclerosis, collagen changes, immune dysfunction and cataracts develop more rapidly in uremia strongly suggest that uremia constitutes a syndrome of accelerated aging, in part due to accelerated Maillard reactions.

Mechanistic studies suggest that glucose is a pentosidine precursor in the extracellular matrix. In contrast, the high correlation between lens pigmentation and pentosidine levels in absence of diabetes suggests that ascorbate oxidation products are pentosidine precursors in the lens and that impaired cellular redox state precedes pentosidine formation. Depending on the turnover rate of the protein, pentosidine levels range from 5-250pmol/mg. Such levels may be by themselves too low to impact on the biology of age-related modifications. However, pentosidine represents the tip of the iceberg of modifications by the Maillard reaction, and other more important modifications are expected to be found *in vivo*.

O 019 ADVANCED GLYCATION ENDPRODUCTS: A MECHANISM FOR AGE-DEPENDENT PERTURBATION OF MONOCYTE AND ENDOTHELIAL CELL FUNCTION. Ann Marie Schmidt, Shi-Du Yan, Yu Shan Zou, Jerry Brett, and David Stern. Dept. of Physiology, Columbia University, College of Physicians and Surgeons, New York, NY 10032

When proteins are exposed to aldose sugars, they undergo nonenzymatic glycation and oxidation. The ultimate products of these interactions are a heterogeneous class of structures termed advanced glycation endproducts (AGEs). These glycated proteins accumulate in the vasculature and tissues with normal aging, and at an increased rate in diabetes. AGEs have been linked to the pathogenesis of microvascular disease and to the accelerated atherosclerosis observed in patients with glucose intolerance. An important potential means through which AGEs exert their effects in vivo is via interaction with cellular receptors. We have characterized cell-associated AGE binding proteins: an integral membrane protein which is a new member of the immunoglobulin superfamily (RAGE= Receptor for AGE), and a lactoferrin-like polypeptide (LF-L). The cell surface binding site for AGEs is comprised of a high affinity, non-covalent complex of RAGE and LF-L. RAGE is expressed by endothelial cells (ECs) and mononuclear phagocytes (MPs) in culture, where it appears to mediate the binding of AGEs. In the tissues, RAGE is expressed by ECs, smooth muscle, cardiac myocytes, cells in the alveolar wall, and neural tissue. The latter includes peripheral nerves (especially in the autonomic nervous system), motor neurons in the spinal cord and a population of cortical neurons. Consistent with these data, RAGE is present in cultured PC12 cells, and, following nerve growth factor-induced neurite outgrowth, RAGE is distributed over their cellular processes. In a pathologic setting, RAGE has been found to mediate the increased adherence of diabetic red cells to endothelium. In addition, incubation of ECs with AGEs leads to increased monolayer permeability, thrombogenicity, and it alters cell growth.

Infusion of AGEs into mice leads to an initial rapid phase of clearance which is delayed/blocked by pre-infusion of anti-RAGE IgG or a truncated soluble form of RAGE (sRAGE). Studies in cell culture confirm that sRAGE blocks AGE interaction with ECs and MPs, and in vivo, sRAGE prevents AGE-mediated induction of PDGF A chain mRNA in the kidney. These data suggest that sRAGE is a reagent capable of dissecting the contribution of AGE-RAGE interaction in complex situations.

Since RAGE is present on MPs, it could potentially mediate removal of AGEs if these cells could gain access to sites of AGE deposition in the tissues. Soluble AGEs induce MP chemotaxis dependent on interaction of glycated proteins with RAGE: anti-RAGE IgG and sRAGE block AGE-mediated MP migration. In contrast to soluble AGEs, when these glycated proteins are immobilized on a surface, they slow MP migration in response to other stimuli. This is due to RAGE engaging immobilized AGEs, resulting in decreased movement as shown in phagokinetic track assays. Interaction of AGEs with ECs and MPs leads to their activation in part due to AGE-induced activation of the transcription factor NF-kB.

Taken together, these data indicate that AGEs modulate a spectrum of EC and MP properties relevant to the pathogenesis of vascular lesions. The cellular effects of AGEs are mediated, in large part, through their cell surface binding site, RAGE/LF-L complex. Future studies utilizing reagents which specifically antagonize AGE-cell surface interactions, and which delineate the regulation and consequences of RAGE binding to its ligand are underway to determine the importance of this pathway as a significant contributor to the pathogenesis of vascular lesions.

Genetics of Age-Related Diseases

O 020 CLINICAL, GENETIC, AND PATHOPHYSIOLOGIC ASPECTS OF WERNER'S SYNDROME ("PROGERIA OF THE ADULT"). G.M. Martin, G.D. Schellenberg, E.M. Wijsman, J. Nakura, T. Miki, K. Kamino, T. Ogihara, C. E. Yu, J. E. McKay, J. Oshima, K.I. Fukuchi, R.J. Monnat Jr., C. M. Disteche, S. Edelhoff, G. C. Burmer, M. Chang, J. B. Sweasy, L. A. Loeb, M. Poot, H. W. Hoehn, and the International WRN Patient Registry Group. Universities of Washington, Osaka, and Wurzburg.

A clinical classification has been developed that differentiates patients according to the strength of the evidence for the diagnosis. Details are available from GMM (FAX 206 685-8356). Qualitatively similar results, however, have been obtained with data analyzed from "definite" vs "definite + probable" diagnostic subsets, confirming previous assignments to 8p12 (Goto et al, Nature, 355:735, 1992; Schellenberg et al, Lancet 339:1002, 1992). Linkage analysis using Japanese (18), Caucasian (7), Hispanic (1), and American black (1) families give maximum LOD scores of 9.02 (θ = 0.01) for D8S87. Multipoint analysis indicates that the most likely location for WRN is between D8S87 and ANK. Several lines of evidence support the hypothesis that homozygosity at the WRN locus results in a mutator phenotype. One candidate locus consistent with this hypothesis, DNA pol-β has been ruled out. The discovery of a cell cycle defect in lymphoblastoid cell lines (Poot et al, Exp. Cell Res., 202:267-273) should permit more focused biochemical studies in these readily available cell cultures. (Supported by grants from the Japanese Ministry of Health, the National Institute on Aging, and Deutsche Forschungsgemeinschaft).

O 021 GENETICS OF LATE-ONSET ALZHEIMER'S DISEASE: WHEN DOES A TRAIT BECOME A DISEASE? Allen D.Roses[1], Ann Marie Saunders[1], Warren Strittmatter[1], Donald Schmechel[1], Sally Joo[1], Barbara Rosi[1],Barbara Crain[1], Christine Hulette[1], Dmitry Goldgaber [2], Margaret Pericak-Vance,[1] [1] Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Duke University Medical Center, Durham, NC 27710, [2] Department of Psychiatry, State University of New York at Stony Brook, Stony Brook, NY.

There is locus heterogeneity of Familial Alzheimer's Disease (FAD). Early-onset (average age of onset <60 years) FAD segregates as an autosomal dominant trait and there are at least three loci: 1) specific, rare mutations of the amyloid precursor protein (APP) located on chromosome 21; 2) robust linkage of chromosome 14 markers in most families previously thought to be on chromosome 21; and 3) an undetermined locus (not on chromosomes 14, 19, or 21) for Volga-German families that may be due to a specific founder effect. Using the Affected Pedigree Member method of Weeks and Lange, there was highly significant genetic association of several contiguous genetic markers on chromosome 19q with late-onset FAD families. When standard likelihood methods were applied using only the data from affected individuals, a multipoint LOD score of 4.6 was found. Addition of age adjusted data from non-affected individuals lowered the LOD score, suggesting that an autosomal dominant model for these data may not be correct. Multiple locus modelling was then initiated for genetic analyses of age dependent disease. Apolipoprotein E (APOE) is a gene located within the genetic association region at chromosome 19q13.2. Independent ß-peptide (ß-amyloid, ßA4) binding studies identified differences in ApoE binding in CSF from AD patients and controls. Immunochemical studies using ApoE antibodies stained neuritic plaques, neurofibrillary tangles and vascular amyloid in brain tissue from AD patients. APOE-E4 allele was significantly associated with late-onset FAD compared to aged controls (0.50 ± 0.06 and 0.16 ± 0.03, p = 0.014). This association was examined in sporadic AD patients who were diagnosed as probable AD as well as a large series of autopsy diagnosed sporadic AD patients. A highly significant association with APOE-E4 was found in sporadic AD. The implications for functional involvement of ApoE isoforms in the pathogenesis of AD and the potential role of APOE-E4 as a risk factor or susceptibility gene will be discussed.

O 022 GENETIC ANALYSIS OF THE CHROMOSOME 14 EARLY-ONSET FAMILIAL ALZHEIMER'S DISEASE LOCUS, Gerard D. Schellenberg[1], Thomas D. Bird[2], Ellen M. Wijsman[3], Harry T. Orr[4], Ellen Nemens[1], June A. White[5], Lori Bonnycastle,[1], Ma. Elisa Alonso[6], Leonard L. Heston[7], and George M. Martin[8], [1]Neurology, Department of Medicine, University of Washington, Seattle, [2]Division of Neurology, Veterans Affairs Medical Center, Seattle, [3]Division of Medical Genetics, Department of Medicine, University of Washington, Seattle, [4]Department of Laboratory Medicine and Pathology and the Institute of Human Genetics, University of Minnesota, Minneapolis, [5]Department of Psychiatry, University of Minnesota, Minneapolis, [6]National Institute of Neurology and Neurosurgery, Mexico City, [7]Washington Institute for Mental Illness Research and Training, Steilacoom, [8]Department of Pathology, University of Washington, Seattle.

Defective genes are responsible for at least some cases of Alzheimer disease (AD). Numerous families have been described in which AD appears to segregate as an autosomal dominant trait. In a subset of early onset familial AD (FAD) kindreds, mutations in the amyloid precursor protein (APP) gene are responsible for the disease. However, the majority of early-onset FAD families including the Volga German kindreds do not appear to have mutations in the APP gene. Similarly, in most if not all late-onset kindreds, FAD does not appear to be caused by APP mutations. The role of inheritance in the more common late-onset AD is not presently resolved; clustering of late-onset cases in individual pedigrees, family history data from case-control studies, and the concordance rates for twins provide evidence that defective genes may also be responsible for some or possibly all late-onset AD. To identify chromosomal regions harboring FAD genes, the genome was searched using linkage analysis. Markers on chromosome 14 gave highly significant positive LOD scores in a group of 9 autopsy-documented early-onset Volga German kindreds; a Z_{max} of 9.15 (Θ = 0.01) was obtained with D14S43 (14q24.3). One family yielded a LOD score of 4.89 (Θ = 0.0). The chromosome 14 FAD locus does not appear to be confined to a single ethnic group; while most of the families studied were Caucasian, a Japanese family also gave positive LOD scores (Z_{max} = 1.49, Θ = 0.0 for D14S53) for chromosome 14 markers. Genetic analysis indicates the FAD gene is between markers D14S53 and D14S52. D14S42 also yields important information concerning the location of the FAD gene. Results for the Volga German families were either negative or non-significant for markers in this region. FAD is clearly genetically heterogeneous and additional loci remain to be described. Several potential chromosome 14 candidate genes exist. The protease inhibitors AACT and PI and the protease cathepsin G (14 q11) appear to be excluded by the linkage data. Other candidate loci include the c-FOS gene (14q24.3) and the heat shock 70 kd protein (HSPA2, 14q22-q24). The c-FOS protein, when complexed with c-Jun, acts as a transcription factor which binds to AP-1 sites such as those found in the promotor of the APP gene; thus c-FOS may be involved in transcriptional regulation of the APP gene. The HSPA2 gene product is a molecular chaperon potentially involved in protein assembly and degradation and could act in one of the APP processing pathways. In addition, the gene products from both c-FOS and HSPA2 have been implicated in injury response mechanisms in the central nervous system. The possibility also exists that the chromosome 14 locus defines a pathogenetic mechanism which is completely distinct from APP processing. For example, the methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase-formayltetrahydrofolate synthetase (MTHFD) gene (14q24) is required for de novo purine nucleotide biosynthesis; a defect in this gene could compromise either energy metabolism or DNA synthesis or repair. Identification of the chromosome 14 FAD gene will yield important information concerning the mechanism responsible for AD.

Late Abstract

SPIN-TRAP COMPOUNDS ALTER OXIDATIVE EVENTS IN AGING BRAIN, Robert A. Floyd, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

The notion that free radicals and/or oxidative damage plays a critical role in the aging process needs to be critically tested. Newer methods have made it possible to more accurately quantitate free radical flux in tissue and oxidative damage to proteins, lipids and nucleic acids. Utilizing salicylate hydroxylation to assay hydroxyl free radical formation and protein carbonyl content as an index of protein oxidation, we (1) have shown that oxidative damage occurs when blood flow resumes in ischemia treated gerbil brain. Older gerbils are much more sensitive to an ischemia/reperfusion insult (IRI) than younger animals (2). In an attempt to trap free radicals produced in brain we discovered that certain spin-trapping compounds protected gerbils if they were given either before or within a short time, up to 1 hour, after an IRI. Chronic low level administration of the spin-trap α-phenyl tert-butyl nitrone (PBN) for 14 days to older gerbils caused a monotonic decrease in the age-associated elevated levels of oxidized protein and a concomitant increase in the depressed levels of glutamine synthetase (GS) and alkaline protease (3). Alkaline protease catalyzes the break down of oxidized protein and GS, an enzyme whose activity is very sensitive to oxidative damage, metabolizes glutamate to glutamine. Cessation of PBN administration causes a subsequent rebound in the levels of oxidized proteins, GS and alkaline protease to those prior to PBN administration (3). The simplest interpretation of these data are that PBN is reacting with and quenching free radicals thus reducing the flux of radicals and reestablishing the oxidative stress balance that occurred in younger animals. The data also indicate that the oxidative damage set-point has changed with age. The reasons for this age-associated change must be under genetic control.

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Apoptosis

O 100 **GENETIC CONTROL OF PROGRAMMED CELL DEATH IN *DROSOPHILA*** J. Abrams, K. White, M. Grether, K. Farrell, L. Young and H. Steller, Howard Hughes Medical Institute, Department of Brain and Cognitive Sciences and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Programmed cell death is a fundamental aspect of metazoan development. To investigate this regressive process in *Drosophila*, we have analyzed the ultrastructure and patterns of cell death during embryogenesis. Cell death occurs by apoptosis and although a predictable distribution of dying cells is observed any given stage the exact numbers and position of dying cells are not invariant. We screened for mutations that affect this process and identified one genomic region which is required for all embryonic cell deaths. Embryos deleted for this locus lack all dying cells during each stage of embryogenesis and have excessive numbers of cells in the nervous system. In addition, the macrophages which engulf dying cells are unusually small and devoid of internalized necrotic material in mutant embryos. Upon exposure of mutant embryos to X-irradiation, fluorescently labelled cells are observed indicating that cellular degeneration can still be induced. The phenotype of these chromosomal deletion stocks thus defines a genetic function that is crucial for the control or the execution of programmed cell death. A detailed genetic and molecular analysis of the obligate region is now in progress.

O 101 ACTIVATION OF APOPTOSIS BY *MYC* IN MYELOID PROGENITOR CELLS IS DOMINANT TO THE SUPPRESSION OF APOPTOSIS BY INTERLEUKIN-3 OR ERYTHROPOIETIN, David S. Askew[1] and John L. Cleveland[2]. Department of Biochemistry[2], St. Jude Children's Research Hospital, Memphis, TN 38105 and Dept. of Pathology and Laboratory Medicine[1], University of Cincinnati Medical Center, Cincinnati, Ohio 45267-0529.

The c-*myc* gene plays a central role in the regulation of growth and differentiation of hematopoietic progenitor cells. Recent evidence has also implicated this gene in the activation of apoptosis signalling pathways in hematopoietic cells. C-*myc* is an immediate early response gene to interleukin-3 (IL-3) stimulation of IL-3-dependent myeloid progenitor cell lines, and levels of *c-myc* rapidly decrease following removal of ligand. We have previously shown that c-*myc* is a potent inducer of apoptosis if is inappropriately expressed in myeloid progenitor cells in the absence of IL-3. Since IL-3 signalling involves pathways which suppress apoptosis, analysis of the effects of constitutive c-*myc* expression in the absence of IL-3 is therefore complicated by the fact that IL-3 deprivation removes this apoptosis-suppressing activity. We were therefore interested in examining the consequences of c-*myc* deregulation in situations where hematopoietic cells undergo G1 arrest in the presence of hemopoietins which are known to promote cell survival and suppress apoptosis. To address this we chose to study two forms of growth arrest which occur in an IL-3-dependent myeloid progenitor cell line: (1) Density-dependent G1 arrest in the presence of IL-3, and (2) G1 arrest in the presence of erythropoietin (Epo). The results demonstrate that activation of apoptosis by *MYC* is dominant to the suppression of apoptosis by either IL-3 or Epo. Down-regulation of c-*myc* expression is therefore required for cells to maintain viability in a G1 state, even in the presence of growth factors which suppress apoptosis.

O 102 STRONG CORRELATION BETWEEN VIRAL RESISTANCE AND APOPTOSIS IN INSECT CELLS PERSISTENTLY INFECTED WITH THE HZ-1 BACULOVIRUS, Yu-Chan Chao, Jin-Chin Lee, Hong-Hwa Chen, and Huang-Liang Wei. Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, R. O. C. 11529.

Resistance to viral superinfection in persistently infected eukaryotic cells is a well-known though poorly understood phenomenon. By using Hz-1 baculovirus and fall armyworm (*Spodoptera frugiperda*, Lepidoptera, Noctuidae) cells as a model, we found that this refractoriness to superinfection was closely related to apoptosis of the host cells. Viral superinfection of persistently infected cells induced apoptosis which occured much earlier than the maturation of the infecting virus. The induction of such early cell death correlated closely with a drastic decrease in the virus titer released from the persistently infected cells. This provides the first example of a direct link between apoptosis and viral resistance in host cells in nature.

O 103 PROGRAMMED CELL DEATH AT METAMORPHOSIS: INDUCTION OF MUSCLE-SPECIFIC PROTEASE GENE EXPRESSION IN THE MOLLUSC *HALIOTIS RUFESCENS*. Bernard M. Degnan and Daniel E. Morse. Marine Biotechnology Center and Department of Biological Sciences, University of California, Santa Barbara, CA 93106.

We have identified a protease gene transcriptionally induced in a tissue developmentally programmed to die. In animals undergoing metamorphosis, there is a dramatic reprogramming of developmental pathways, resulting in global morphological changes of the animal body plan. Programmed cell death is an integral component of this process, degrading redundant larval tissues and allowing sculpting of the adult from the larval body form. Metamorphosis of larvae of the gastropod mollusc, *Haliotis rufescens*, can be induced in culture, providing a uniquely tractable model system for analysis of gene regulation controlling this developmental event. The ventral larval retractor muscle is a specialized larval tissue that atrophies during metamorphosis. *In situ* hybridization and northern analysis reveals a dramatic induction of serine protease gene transcription in the this muscle ca. 24 h after the larvae are induced to begin metamorphosis by an exogenous morphogen. This transcript continues accumulating for at least four days. F-actin is detectable in the ventral retractor muscle up to 42 h after induction to initiate metamorphosis and is solublized, and presumably degraded, by 70 h. These results indicate that active transcription and destruction of myofibrils is occurring simultaneously. Transcripts of this gene are not detected in other larval retractor muscle cells that are destined to be recruited into the adult muscle systems. It is likely that the serine protease catalyzes digestion of the targeted muscle fibrils, and thus directly mediates their apoptosis.

O 104 PROGRAMMED CELL DEATH IN HETEROKARYONS: A STUDY OF THE TRANSFER OF APOPTOSIS BETWEEN NUCLEI. Bruno Dipasquale and Richard J. Youle, Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892-0010.

Thymocytes undergoing apoptosis induced by dexamethasone showed nuclear refractility changes under Nomarski optics that correlated precisely with internucleosomal DNA degradation and were prevented by cycloheximide. When heterokaryons between thymocytes and 9L or NIH3T3 cells were examined, 99.75% of the nuclei in heterokaryons followed the original and distinct fate characteristic of the parental cells. Thymocyte nuclei proceeded to undergo apoptotic cell death, whereas the 9L and NIH3T3 nuclei in the heterokaryons did not show the morphological changes of apoptosis or any DNA cleavage on gels, and remained viable and mitotic. Cycloheximide prevented the induction of apoptosis in thymocyte nuclei in the heterokaryons. An excess of up to seven thymocyte nuclei undergoing programmed cell death in a heterokaryon did not detectably damage the 9L nucleus and an excess of six 9L nuclei did not protect the thymocyte nucleus from apoptosis. The proposed model stating that programmed cell death results from de novo synthesis of death causing gene products is difficult to reconcile with our findings. A cell-type specific activity, present in thymocyte nuclei before induction of apoptosis with corticosteroids and unable to diffuse between nuclei, appears to be responsible for DNA fragmentation. Our results also show that nuclear disintegration is the trigger of thymocyte death and not a consequence of cell death due to another mechanism.

O 105 GLUTAMATE INDUCES APOPTOTIC CELL DEATH IN PRIMARY CULTURES OF THE NERVOUS SYSTEM. Françoise Finiels-Marlier, Bruno Dipasquale *, Ann M. Marini, Steven Paul and Richard J. Youle *, Section on Molecular Pharmacology, Clinical Neuroscience Branch, National Institute of Mental Health and * Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892-0010.

Primary cultures of cortical neurons and cerebellar granule cells are susceptible to the toxic effect of glutamate. We studied these neurons following exposure to toxic concentrations of glutamate using Nomarski optics which identifies apoptotic cells as round and highly refractive bodies. The control level of apoptotic refractive cells in control cultures of cortical neurons was 3-5%, whereas in cerebellar granule cells it was 20-30%. The percentage of apoptotic refractive cells increased up to 15-20% in cortical neurons and up to about 50% in cerebellar granule cells following glutamate (150 and 30μM respectively) exposure at 2 and 4 hours. Apoptotic refractive changes were inhibited by cycloheximide (1μg/ml) or the non-competitive NMDA receptor antagonist MK801 (1μM); both reduced the percentage of apoptotic refractive cells to control levels. In thymocytes the apoptotic refractive changes identified by Nomarski optics correlate with internucleosomal DNA degradation. Therefore, we extracted the DNA from glutamate-treated cultures, but found no evidence of internucleosomal DNA degradation. Our findings show that the early nuclear changes detected using Nomarski optics, characteristic of apoptotic cell death, result from glutamate exposure of the cultured neurons. However, we have not been able to detect the DNA degradation pattern that other neurotoxins induce in cultured neurons (ref. Dipasquale et al. 1991, Biochem. Biophys. Res. Commun. 181:1442-1448).

O 106 THE NEUROPROTECTIVE DRUG L-ACETYLCARNITINE DELAYS APOPTOSIS IN NERVE-LIKE CELLS

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P19 teratoma cells differentiate to neural-like cells in the presence of retinoic acid. If they are plated in N2 synthetic, serum-free, medium without being exposed to retinoic acid, they die within 48-72 h, by apoptosis. We have used this model to study the mechanism of action of L-acetylcarnitine. This endogenous molecule has been successfully employed as a drug retarding Alzheimer's disease progression (A. Spagnoli et al. Neurology 41, 1726-1732, 1991). Many pharmacological actions have been reported for this compound. In our model the addition of 100 μM L-acetylcarnitine to the N2 medium, at the time of plating, enhances cell survival and inhibits DNA fragmentation and nuclear condensation (Galli & Fratelli, Exp. Cell Res., in press). In the same cells, the oxidant tert butyl hydroperoxide, whose activity has been verified by measuring reduced glutathione levels, causes an extensive cell death that does not involve nuclear condensation and fragmentation, and has the characteristics of necrosis. This cell death does not appear to be inhibited by L-acetylcarnitine. Therefore the protective action of L-acetylcarnitine seems to be specifically on apoptotic, and not on necrotic cell death. The effect of L-acetylcarnitine treatment on the expression of some genes involved in apoptosis will also be presented.

The data presented here shed a new light on the mechanism of protection exerted by L-acetylcarnitine in neurodegenerative diseases.

O 107 GENES EXPRESSED IN COMMON DURING MAMMARY GLAND INVOLUTION AND DURING CASTRATION-INDUCED PROSTATE REGRESSION. Robert Friis, Wolfgang Bielke, Guo Ke and Susanne Saurer, Laboratory for clinical experimental Research, University of Berne, Tiefenaustr. 120, CH 3004 Berne, Switzerland.

The goal of our invesigation has been the isolation of additional genes which participate functionally in the programmed cell death of mammary epithelium taking place following lactation (Strange et al., Development 115: 49). In our recent experiments, we have attempted to discriminate between apoptotsis-specific events, and the tissue remodelling which occurs in the mammary gland at the same time, using a coincidence screening approach taking advantage of the similarities in programmed cell death of epithelium observed in mammary gland involution and prostate regression following castration. This coincidence screening method was employed to detect clones in a lambda cDNA library prepared from 1-2 day involuting mouse mammary gland mRNA. Two probes were employed in the differential screening of this library. Both probes were prepared using a subtraction protocol: Probe 1 was 2-3 day involuting mammary gland 1st strand cDNA which had been subtracted against an excess of pregnant and lactating mammary gland mRNA, dA-tailed and amplified using PCR. Probe 2 was 2-3 day castration-induced, regressing prostate 1st strand cDNA which had been subtracted against an excess of mature male prostate mRNA, tailed and amplified using PCR. Lambda phage plaque lifts were differentially screened with the two probes in parallel, and only those plaques were isolated which were positive with both probes.

This protocol has added to our collection of genes known to be expressed in a mammary involution-specific fashion. Sequencing and characterization of expression of several previously un-characterized genes is in progress. More than one dozen functionally characterized genes, including those encoding a leucine zipper protein, the 24p3 SV40-induced protein, the WD NM1 non-metastatic protein, sulfated glycoprotein-2, osteopontin, etc. will be discussed in terms of their specificity for expression in mammary gland involution and/or prostate regression.

O 108 A SENSITIVE NON-RADIOACTIVE METHOD FOR DETECTING APOPTOTIC OLIGONUCLEOSOMAL FRAGMENTS FROM ELECTROPHORETIC AGAROSE GELS, Meredith L. Howell, Carlos J. Carrera, and Dennis A. Carson, Department of Medicine and the Sam and Rose Stein Institute for Research on Aging, University of California, San Diego, La Jolla, CA, 92093-0663

An understanding of the biochemical mechanism of apoptosis has been hampered by the lack of a sensitive and visual assay for detection of the endonuclease-induced DNA fragmentation that is closely associated with this process. We have developed such an assay that utilizes Southern blot hybridization of the fragmented DNA with a non-radioactive oligonucleotide probe specific to human genomic DNA sequences. Initially, endonuclease-cleaved DNA released from Triton-X-100 permeabilized cells is separated from full length chromosomal DNA by centrifugation. Samples are then electrophoresed, pressure-blotted onto a synthetic positively-charged membrane, and hybridized with probe. The hybridized DNA is detected by an antibody-conjugate to a modified digoxigenin nucleotide base incorporated into the DNA probe. The signal from this DNA-antibody complex is then visualized by chemiluminescence and autoradiography and can be quantitated by scanning densitometry. The strength of this hybridization technique is its sensitivity. The characteristic nucleosomal banding pattern of the fragmented DNA can easily be visualized in as few as 10,000 human peripheral blood lymphocytes treated with the chemotherapeutic compound 2-chlorodeoxyadenosine, a potent inducer of apoptosis. This represents well over a hundred-fold increase in sensitivity when compared to DNA detection by ethidium bromide staining of agarose gels. Estimation of the amount of fragmented DNA using this technique yields similar results to DNA quantitation by the diphenylamine assay.

O 109 **PROGRAMMED NEURONAL CELL DEATH IN PC12 CELLS PRIMED WITH NERVE GROWTH FACTOR**, T. Koike and S. Tanaka, Department of Natural Science, Saga Medical School, Nabeshima, Saga 849, Japan

Target-derived neurotrophic factors have a pivotal role in regulating neuronal death during development or after target removal or axotomy. Molecular processes triggered by trophic factor deprivation are yet to be clarified. We have previously shown that PC12 cells undergo degeneration after withdrawal of nerve growth factor (NGF) only when they have been treated with NGF (Soc. Neurosci. Abstr.17: 228.1991; Prog. Neuro-Psychopharmacol. & Biol-Psychiat. 16: 95-106, 1992). Using these cells as a model system, we are currently characterizing degenerative processes that occur in response to acute NGF withdrawal. PC12 cells treated with nerve growth factor (50ng/ml) for 10-14 days underwent massive degeneration characterized by disintegration of neurites, decrease in the ATP content to less than 50% , which occurred in conjunction with an increase in release of the cytoplasmic enzyme, lactate dehydrogenase, LDH into medium (40-45 % of the total activity by 35h after deprivation). Eighty five to ninety % of LDH release was blocked by treating NGF-deprived cells with actinomycin D (0.3μM) or cordycepin; the residual release was due to neurite degeneration. Thus, NGF deprivation-induced cell death of PC12 cells occurs in a transcription-dependent manner, while serum deprivation-induced PC12 cell death has been reported to occur via a transcription-indepenedent mechanism. The cell death was completely prevented by chronic depolarization with high potassium (>35mM), CPT-cAMP (>0.1mM), acidic and basic FGF (10ng/ml), but other growth factors including EGF, PDGF,TGF or cholinergic agonists or TPA were ineffective. Upon NGF deprivation, tyrosine dephosphorylation of major proteins, p120, p90, p73, p63 occurred within 30 min after NGF withdrawal. Assessing the activities of protein kinases bound to nitrocellulose-blotting membranes allowed us to identify activities being gradually decreased upon NGF withdrawal, which appeared to require RNA and protein synthesis. Finally, DNA fragmentation occurred concomitantly with cell death. These results demonstrate the feasibility of differentiated PC12 cells as a model for studying neuronal apoptosis *in vitro*.

O 110 PULSE FIELD GEL (PFG) ELECTROPHORETIC STUDY OF CELL GENOME BREAKDOWN IN RELATION TO APOPTOSIS, Ludmila B. Kokileva, Marianna Sikorska and Roy Walker, Molecular Cell Biology Group, Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ont. K1A OR6.

The knowledge of cell genome breakdown is needed to elucidate the mechanism of apoptosis. PFGE revealed strongly ordered pattern of endogenous DNA degradation in intact thymocytes and isolated rat liver and thymocyte nuclei. The advanced DNA cleavage to oligo- and mononucleosomes was preceded by an initial stage of endogenous DNA breakdown to large fragments (50-600 kbp). Conditions which induce apoptosis, t.e. VM-26, dexamethasone or irradiation induce the same pattern of endogenous DNA degradation. Separation of initial and advanced stages of DNA degradation was achieved in nuclei or intact thymocytes deprived of divalent cations. ZnCl2 completely inhibited the DNA cleavage to ladder but did not affect the generation of large DNA fragments. This shows that the initial endonucleolytic activity is different from that of Ca/Mg-endonuclease. The experimental data with VM-26 points to the possible involvement of topoisomerase II. The role of Ca/Mg endonuclease action in the pattern of genome breakdown has been demonstrated (50 kpb DNA to ladder).

O 111 DNA FRAGMENTATION INDICATIVE OF APOPTOSIS IN BRAIN FOLLOWING ISCHEMIA, John P. MacManus, Ingrid Rasquinha, Edward Preston and Alastair M. Buchan, Institutes for Biological Sciences and Biodiagnostics, National Research Council of Canada, Ottawa, K1A 0R6, and Loeb Institute, Civic Hospital, Ottawa, Canada.

Gene-directed cell death or apoptosis has been demonstrated in brain during development and also following treatment of brain cells with either glutamate or 1-methyl-4-phenylpyridinium. Although excessive glutamate release is implicated in brain injury after ischemia, a morphological study failed to find evidence of apoptosis. Herein we present biochemical evidence of internucleosomal cleavage of brain DNA indicative of apoptosis following ischemia.

Neocortex, hippocampus and striatum were sampled after rats had undergone one of the following ischemia protocols: 2 vessel occlusion plus hypotension (2VO), 4 vessel occlusion (4VO) or middle cerebral artery occlusion (MCAO). In ethidium bromide stained agarose gels, DNA laddering was observed in the striatum 24, 48 and 72 hours following 2 or 4VO. A 100-fold increase in detectability of fragmented DNA was achieved by 3'-end-labeling with alpha-^{32}P-dideoxy nucleotides. By gel autoradiography this procedure showed greater DNA damage in the CA1 layer of the hippocampus than in CA3 in both 2 and 4VO models. Little DNA fragmentation was observed in the neocortex. A quantitative measure of DNA breaks was possible by counting Cerenkov radiation in gel lanes. The number of DNA breaks in the striatum at 48 hours post-ischemia was shown to increase as the duration of the 2VO period was increased (8, 12 and 16 minutes). It was autoradiographically shown that 24 hours after temporary focal ischemia (MCAO), the amount of DNA cleavage in the core of the neocortical injury zone exceeded that in the penumbra. Radiation counting also showed that the number of DNA breaks increased with the duration of MCAO (0.5 to 3 hours).

These methods have demonstrated a correlation between occurrence in vivo of apoptotic DNA fragmentation and the known regional and temporal patterns of neuronal death in three established rat models of ischemic brain damage. The quantitation of the damage to DNA showed it to be dependent on the duration of the ischemic episode. We conclude that ischemia can cause apoptosis in brain cells in vivo.

O 112 **Deregulation of Apoptosis in Preneoplastic Cell Lines is Linked to Immortalization and Loss of Tumor Suppressor Gene Function.** Gloria A.Preston and J. Carl Barrett. Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

At present, it appears that the regulatory genes of apoptosis are familiar genes which normally function to regulate cell proliferation, but which are commonly found to be abnormally regulated in neoplasms.

It is possible that deregulation of cell proliferation by immortalization and/or loss of tumor suppressor gene function could result in a concomitant alteration in apoptotic susceptibility. To address this possibility the Syrian hamster embryo (SHE) cellular system characterized in our laboratory was utilized. Cell lines which represent different stages of the neoplastic process were placed under various adverse conditions and the mechanism of death was determined.

We report that normal, diploid SHE cells were resistant to apoptosis under all conditions evaluated, whereas the immortal cell lines at early passages showed a high susceptibility to apoptosis indicating that the once suppressed apoptotic response is now readily active. It is reasonable to assume susceptibility is coupled with the acquisition of immortality and that these events are interrelated. Through the acquisition of further genetic defects, eg., loss of tumor-suppressor gene function, the sup- cells are successfully 'rescued' from apoptosis. The cells continue to divide even in adverse conditions until death by necrosis or until they develop resistance through mutation or gene amplification. These results provide indirect support for the hypothesis that gene products involved in neoplastic progression are the mediators for apoptosis.

O 113 APOPTOSIS IN *MYC*-TRANSFORMED RAT FIBROBLASTS, Michael Small, Andrew Wagner*, Sridevi Naidu, Koichi Iwata, Elizabeth Ortiz, and Nissim Hay*, Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ 07103, and *The Ben May Institute, Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637

The c-*myc* proto-oncogene has been implicated in the regulation of normal and malignant cell growth and in the genesis of a variety of human tumors. Recently, it has been demonstrated that, under appropriate conditions, the proliferation of *myc*-transformed cells is accompanied by substantial cell death which is dependent on c-*myc* expression and attributable to programmed cell death (apoptosis). We have assessed this phenomenon in normal and *myc*-transformed rat fibroblasts with regard to morphological changes as well as the electrophoretic analysis of low molecular weight DNA for evidence of nucleosomal laddering. We have shown that: (i) Apoptosis is rapidly induced in *myc*-transformed rat fibroblasts when they are deprived of serum growth factors; apoptosis occurs to a much lesser extent in nontransformed cells under similar growth conditions. (ii) Serum deprivation does not appear to promote apoptosis in cells transformed by the human c-*fos* proto-oncogene. However, apoptosis occurs in cells transformed by *myc* or *fos* when maintained at high density in complete medium containing normal levels of serum (iii) Ectopic expression of the the proto-oncogene *bcl2*, previously shown to inhibit apoptosis in hematopoietic systems, specifically blocks apoptosis induced by constitutive *myc* expression. Our results suggest that apoptosis may serve a protective role in preventing tumorigenicity elicited by deregulated Myc expression. This protective mechanism is abrogated by Bcl-2, and therefore may explain the synergism between Myc and Bcl-2 observed in certain tumor cells.

O 114 FURTHER CHARACTERIZATION OF PROTEASE INHIBITOR TREATED APOPTOTIC THYMOCYTES, V.M. Weaver*, L. Kokileva*, B. Lach°, P.R. Walker* and M. Sikorska*, *Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A OR6; °Ottawa Civic Hospital, Ottawa, Ontario.

Internucleosomal DNA cleavage, mediated by a calcium regulated endonuclease, has been defined as the hallmark of apoptosis. We previously demonstrated that serine protease inhibitors (SPI) prevented apoptotic internucleosomal DNA cleavage and decreased cell volume in thymocytes treated with either 1 μM dexamethasone or 5 μM teniposide-VM-26 (topoisomerase II inhibitor) (Weaver, V.M. et al., Mol. Biol. Cell 1992 Suppl. 3: 328a). Here we report a further morphological and biochemical characterization of the effects of these inhibitors on indices of apoptosis in thymocytes. Electron microscopy of dexamethansone treated thymocytes exposed to SPI revealed a decreased preponderance of apoptotic electron dense small cells and an absence of nuclear pyknosis when compared to dexamethasone treated controls. Despite these differences, the SPI treated cells demonstrated several key morphological features of apoptosis including: plasma membrane blebbing, organelle disorganization and partial chromatin condensation and rearrangement. There was no evidence of cellular or organelle swelling typical of necrosis in inhibitor treated cells. Further, despite the absence of internucleosomal DNA cleavage, high molecular weight (hWM) DNA cleavage was evident as assessed by conventional gel electrophoresis of DNA from these cells. Additional kinetic studies, utilizing pulse-field gel electrophoresis of DNA, demonstrated the occurrence of ongoing hMW DNA cleavage (>50 kb) induced by dexamethasone or VM-26 that was preventable by protein synthesis inhibitors but not by SPI. In unison these results suggest apoptosis may progress despite the absence of internucleosomal DNA cleavage and imply a role for serine proteases in endonuclease mediated DNA cleavage.

O 115 **HUMAN TRPM-2: ISOLATION AND MOLECULAR CHARACTERIZATION.** **P.Wong[1,2], J.Lakins[1], D.Taillefer[1], T.Putilina[2], G.Chader[2], M.Tenniswood[1]. Department of Biochemistry, University of Ottawa, Canada[1] and the National Eye Institute, NIH, Bethesda[2].**

Human TRPM-2, also known as clusterin, CLI, and SP-40,40, exists as a single gene locus. Increased TRPM-2 expression has been observed in a number of human degeneration disorders including RP and Alzheimer's disease. In addition the TRPM-2 gene has been shown to be an apoptosis inducible gene.

We present here the characterization of the human TRPM-2 gene which is 16.9 Kbp in size and consists of 9 exons and 8 introns. The expressed sequence is 1.6 kbp and is expressed in a wide range of normal human tissues. Southern analysis of human genomic DNA confirms that human TRPM-2 exists as a single gene locus and, based on fluorescent *in situ* suppression hybridization results, is located on the short arm of chromosome 8.

The results of zooblot analysis show that the TRPM-2 sequence is highly conserved. Comparison of published orthologous TRPM-2 sequences allowed the identification of specific regions of the expressed gene sequence which are highly conserved (greater than 70% orthology). With respect to sequence orthology it appears that the mature TRPM-2 gene product is bipartite in nature with one end of the molecule showing high degrees of cross species similarity and the other displaying a higher degree of species specificity.

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O 116 *IN SITU* DETECTION OF DNA FRAGMENTATION ASSOCIATED WITH CELL LOSS IN THE THYMUS AND THE BRAIN, Katherine A. Wood, Bruno Dipasquale and Richard J. Youle, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892, USA

To date there is scant evidence for apoptosis associated with *physiologic neuron* death, largely due to the lack of appropriate detection methods. A common biochemical event associated with apoptosis is DNA fragmentation, characteristically nucleosome size multimers of DNA detected by agarose gel electrophoresis. Therefore analysis of apoptosis is usually contingent upon having high cell numbers and is generally restricted to cells cultured *in vitro*. We have developed a new method for the detection of DNA fragmentation sensitive at the single cell level that is particularly pertinent for the analysis of tissue sections. Examination of DNA fragmentation in frozen sections of the thymus of mice following *in vivo* induction of apoptosis by dexamethasone injection confirms that the method detects apoptosis in individual cells *in situ*. In addition to DNA fragmentation, individual dying cells were characterized by morphology, and by the location of the cell within the gross architecture of the tissue. This new method to detect DNA fragmentation during apoptosis *in situ* should be valuable to study the mechanism of cell death during development in many tissues and in disease. We have used the method to examine the role apoptosis may play in neural development and in neurodegenerative disease.

Toxicity, Cell Senescence, and Stress

O 200 REPRODUCIBLE DATA ON THE MOLECULAR MECHANISMS OF CELLULAR AGING IN VIVO AND IN VITRO CAN ONLY BE OBTAINED FROM CELL POPULATIONS THAT ARE DEFINED BY STEM CELL SYSTEM SPECIFIC METHODS.

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Most data of studies of the molecular mechanisms of cellular aging in vivo and in vitro are obtained from cellbiologically undefined organs, tissues and cell systems. Because of the heterogeneity of the populations and the changes in the composition of different celltypes in cell populations of different in vivo and in vitro age, undefined cell systems can not yield reproducible data. Exceptions are the cellbiologically fully defined nematods in vivo and in vivo and in vitro investigations of the hematopoietic stem cell systems.

Most studies of the molecular mechanisms of cellular aging of fibroblast populations in vitro are undertaken with undefined populations with standardized methodical parameters like cell numbers, cumulative population doublings and time periods of populations in a non-proliferating state. We have standardized our methods on the reproducible changes of the composition of fibroblast populations in vitro by mitotic and postmitotic fibroblast celltypes of differentiation stages in the development of the fibroblasts in the four compartments of the fibroblast stem cell system in vitro. We have tested the methods of several other groups. They all make late mitotic and early postmitotic fibroblasts differentiate abnormally in Phase III. Not one method tested provides the conditions for the cells to differentiate to normal postmitotic fibroblasts of the maturing compartment. Fibroblasts in the so-called Phase III are cellbiological artefacts and can not be regarded as senescent cells. Cellular aging of fibroblasts in the fibroblast stem cell system takes place in the transition period between the late postmitotic terminally differentiated PMF VI in the maturing compartment and PMF VIIa (apoptosis) in the degenerating compartment.

Bayreuther et al., Proc.Natl.Acad.Sci.USA, Vol.85, pp 5112-5116, 1988

O 201 REGULATION OF DNA-SYNTHESIS IN MITOTIC AND POSTMITOTIC WI 38 FIBROBLASTS IN THE FIBROBLAST STEM CELL SYSTEM.

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As a function of the in vitro age the fibroblast cell types in the secondary fibroblast cell line WI 38 develop in one direction along a seven stage cell lineage with the mitotic fibroblasts MF I - II - III in the progenitor compartment, the postmitotic fibroblasts PMF IV - V - VI in the maturing compartment and the postmitotic fibroblast PMF VIIa (apoptosis) in the degeneration compartment of the fibroblast stem cell system. When appropriate methods are applied, postmitotic fibroblasts arise spontaneously at about CPD level 50-60 or can be experimentally induced by treating MF II populations with for example mitomycin C. Cellular aging of the fibroblasts takes place in the transition period between the late terminally differentiated PMF VI and PMF VIIa (apoptosis). Mitotic fibroblasts MF I - II - III have 2C DNA, whilst in postmitotic fibroblasts PMF IV - V - VI the DNA content increases to 4C and 8C. The DNA synthesis was determined by DAPI-flowcytometry, [^{3}H]-methyl-thymidine and BrdU methods. The DNA synthesis can be reduced by incubation of the mitotic and postmitotic fibroblasts with anti-sense c-fos DNA. ELISA experiments show expression of c-fos and c-jun in mitotic and postmitotic fibroblasts. Restriction analysis of the DNA of spontaneously arising and experimentally induced (MMC) postmitotic fibroblasts demonstrates that the pattern of DNA fragments, determined by means of (GTG) $_5$ and (GACA) $_4$, is similar to identical. The fibroblast cell types are characterized by cell type specific [^{35}S]-methionine polypeptide patterns.

Bayreuther, K. et al.: Proc. Natl. Acad. Sci. USA 85,5112-5116 (1988)
Bayreuther, K. et al.: Arch. Gerontol. Geriatrics, Suppl. 3, 47-74 (1992)

O 202 THE ROLE OF PROTEIN SYNTHESIS AND POST-TRANSLATIONAL MODIFICATIONS IN AGEING

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Age-related changes in the functioning of proteins can be due to inefficient protein synthesis, altered pattern of post-translational modifications, and defective pathways of protein turnover. Slowing-down of bulk protein synthesis is a widely recognized biochemical change with age. Out of various components of the protein synthetic machinery, elongation factors appear to play a crucial role in the regulation of protein synthesis during ageing. The activities of the elongation factors may themselves be regulated by post-translational modifications, such as methylation, phosphorylation and ADP-ribosylation. Similarly, the accumulation of abnormal proteins during ageing is considered to be a function of their post-synthetic modifications including oxidation of amino acids, deamidation, racemization, and spontaneous changes in protein conformation.

We have been studying the regulation of protein synthesis at the levels of transcriptional, translational and post-translational changes in elongation factors EF-1α and EF-2 in ageing human fibroblasts and keratinocytes. Furthermore, since EF-1α appears to be a candidate gerontogene because of its life span extending effects reported for transgenic *Drosophila*, currently we are studying various constructs of human EF-1α for expression in transgenic mice to detail any life span changes in the component cells.

O 203 THE GENE RESPONSIBLE FOR WERNER'S SYNDROME MAY BE A CELL GROWTH "CLOCK" GENE

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Werner's Syndrome is a rare autosomal, recessive condition characterised by short stature, a hoarse high-pitched voice, juvenile bilateral cataracts, premature greying of the hair, skin changes, diabetes, atherosclerosis and other diseases characteristic of the elderly. Generally, the condition only becomes apparent between 20 and 30 years of age. Progressive atrophy of the skin and subcutaneous tissue of the distal limbs, precedes characteristic foot ulceration and eventual gangrene. Werner's Syndrome is frequently studied as a model of some aspects of human ageing, but the behavioural changes which are usually associated with old age are seen infrequently. A most striking phenotype of Werner's Syndrome, presumably arising from the same gene defect, is a dramatic shortening of the replicative life-span of dermal fibroblasts in vitro. The finite replicative life-span of human cells in vitro is due to the stochastic loss of replicative ability in a continuously increasing fraction of new-born cells at every generation. Normal human fibroblasts achieve about 60 population doublings in culture, while Werner's Syndrome cells usually only manage to achieve about 20 population doublings. We describe an analysis of the replicative ability of fibroblasts from Werner's Syndrome patients and demonstrate that these cultures usually start off with a good replicative ability, but that they exit apparently irreversibly from the cell-cycle at a faster rate than do normal cells. We conclude that, perhaps, the Werner's Syndrome gene is a "clock" gene controlling the number of times human cells are able to divide before terminal differentiation.

O 204 FAS IS INVOLVED IN A MECHANISM OF T CELL-MEDIATED CYTOTOXICITY

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Programmed cell death, often called apoptosis, massively occurs within the immune system as a component of its normal development. Part of this process seems to involve the Fas cell surface molecule, which can transduce a cell death signal and is not or abnormally expressed in the *lpr* mutant mouse exhibiting a lymphoproliferative disorder. Cell death can also be imparted by the immune system, in particular through T cell-mediated cytotoxicity. Hypotheses as to the undefined and apparently multiple molecular mechanisms of this process range from direct membrane interactions to granule exocytosis.

To investigate some of these mechanisms, we used as target cells on the one hand thymocytes from *lpr* and *gld* mouse mutants and on the other hand L1210 cells transfected or not with the apoptosis-inducing Fas molecule. These independent approaches both led to the conclusion that Fas was involved in the Ca^{++}-independent component of cytotoxicity mediated by at least two sources of T cells, namely non-antigen-specific in vitro activated hybridoma cells, and antigen-specific in vivo raised peritoneal exudate lymphocytes. Thus, in these cases T cell-mediated cytotoxicity involved direct membrane interactions and transduction via Fas of the target cell death signal. From a more general point of view, Fas thus seems causally involved in cell death in both developmental and functional circumstances within the immune system.

O 205 STRESS PROTEINS DO NOT PLAY A ROLE AS ORGAN-SPECIFIC T-CELL AUTOANTIGENS, BUT MAY FACILITATE AUTOANTIGEN-PRESENTATION IN THYROID AUTOIMMUNE DISEASE

B.Grubeck-Loebenstein, K.Trieb, A.Sztankay, H.Lechner and G.Wick, Institute for Biomedical Aging Research of the Austrian Academy of Sciences, 6020 Innsbruck, Austria. Autoimmune thyroiditis (AT) frequently occurs in old age. Thyroid derived polyclonal T cell lines from patients with AT have been shown to respond to recombinant heat shock protein 70 (hsp 70) of various origin as well as to autologous thyroid epithelial cells (TEC). To determine whether one or two types of T-cells were responsible for this response, 130 thyroid derived T-cell clones were screened for their reactivity to recombinant hsp as well as to irradiated autologous TEC, PBMC and EBV transformed B-cells. 5% of the clones responded to recombinant hsp 70, 0.7% to hsp 65, 6% to PMBC and EBV cells and 18% to TEC. Clones which reacted with recombinant hsp or autologous cells were all CD4+. All clones responsive to recombinant hsp could also be stimulated with heat shocked and unshocked PBMC and EBV cells, but did not recognize TEC. In contrast TEC responsive clones did not react with recombinant hsp 70 or hsp 65. Interestingly, the response of the majority of TEC reactive clones could be dramatically increased, when heat shocked TEC were used as stimulator cells. These results demonstrate that T cells specific for hsp 70 or hsp 65 do not recognize their antigen on intact thyroid epithelial cells. They therefore do not seem to play a primary pathogenetic role in the autoimmune thyroid gland. Stress inducible proteins may still be of importance in the autoimmune process, firstly as autoantigens on infiltrating inflammatory cells, and secondly by facilitating the presentation of thyroid specific autoantigen(s) to autoreactive T-cells. This mechanism may be of particular importance in senescent cells, in which stress protein induction may be increased by a multitude of different environmental insults.

O 206 STUDIES OF SENESCENT CELL BEHAVIOR IN RESPONSE TO PDGF UNDER CONDITIONS OF VARYING STRESS, Rosalind A. Grymes, Christine Sawyer, and Maronda V. Brown*, Life Science Division, NASA-Ames Research Center, Moffett Field, CA 94035; *Department of Biological Sciences, Morris Brown College, Atlanta, GA 30314

Spaceflight studies using mammalian cells of diverse lineages have indicated that microgravity-exposed cells are inhibited in their responses to normally stimulatory signals and/or fail to make normal progress along differentiation pathways. Many of these findings have been confirmed and extended using ground-based models. Loss of response capacity and of differentiation potential are also characteristics of senescent cells. We are working with dermal fibroblasts derived from skin biopsies of Werner's syndrome (WS) patients to investigate intracellular signal transduction events that may underlie the abnormalities observed in these cells with respect to platelet-derived growth factor (PDGF) response. WS is an autosomal recessive progeroid syndrome exhibiting changes in skin and connective tissue *in vivo*. Our earlier studies demonstrated that PDGF induction of collagenase type I expression or cell proliferation is markedly absent from WS dermal fibroblast cell strains when compared with normal fetal counterparts. While gravity is a small force to be perceived at the single cell level, intracellular amplification of chemical/enzymatic signals and stuctural/cytoskeletal signals is well-suited to promulgating such small inputs. We are investigating the relationships between perceived intracellular forces and growth factor responses. PDGF treatment of both WS and normal (chronologically aged or fetal) fibroblasts is performed under both static and mechanically active culture conditions. Data regarding PDGF receptor phosphorylation, intermediate filament network organization, and RNA levels (*c-fos, c-jun,* and *collagenase type I*) will be presented.

O 207 HUMAN MITOCHONDRIAL SUPEROXIDE DISMUTASE: A LESS ACTIVE POLYMORPHIC FORM AND TYPE II DIABETES, PARKINSON'S AND ALZHEIMER'S DISEASES, Robert A. Hallewell†‡, Maurice Boissinot*, Michael Johnson†*, and John A. Tainer*, †Biochemistry Department, Imperial College, London; †Atropos Genetic Engineering Inc., San Francisco; *Molecular Biology Department, Scripps Research Institute, La Jolla

Heritable mutations in mitochondrial DNA have been shown to be responsible for two rare forms of type II diabetes (late onset, insulin independent)[1]. The human mitochondrial Mn superoxide dismutase is highly homologous to the bacterial enzyme and *E. coli* mutants lacking this superoxide dismutase activity have a 40-fold increased spontaneous mutation rate[2]. Thus, mitochondrial Mn superoxide dismutase may be an important enzyme protecting mitochondrial DNA from oxidative mutagenesis and individuals with less active forms of the enzyme may be predisposed to mutations in mitochondrial DNA. We have recently described a relatively common polymorphism (Ile58Thr) of the human Mn superoxide dismutase that has greatly reduced activity and stability[3]. The characterization of the native and polymorphic enzyme, and the frequency of the polymorphic form in control, diabetic, Parkinson's and Alzheimer's populations, will be described.

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O 208 **AN AUTOCRINE RESISTANCE FACTOR FROM MELANOMA THAT RESCUES CELLS FROM TOXIC AGENTS.** **Helene Z. Hill, Uwe Schlehaider, Indu Chowdhary and George J. Hill, Departments of Surgery and Radiology, New Jersey Medical School, Newark, NJ.**

Cloudman S91 mouse melanoma cell lines differ in their responses to several toxic agents. S91/amel cells are more sensitive to killing by ionizing radiation and mitomycin C than S91/I3 cells, while S91/I3 cells are more sensitive to killing by UVC than S91/amel cells. We have shown that both cell lines produce a diffusible factor that causes S91/amel cells become less sensitive to the lethal agents but does not affect the survival of S91/I3 cells. Nevertheless, the S91/I3 cells either produce a more effective or a more abundant factor than the S91/amel cells (Hill, Trizna, Ali and Hill, Radiat. Res. 129: 43-47, 1992). The factor produced by heavily irradiated cells (HRCells) or in conditioned medium is effective when added to the target cells as long as 4 days after irradiation. The longer the factor is present during the incubation of the irradiated colony-forming units before it is removed, the greater the survival. The presence of the factor under high salt post-irradiation conditions which inhibit repair enzymes does not alter the extent of this inhibition. The factor (or factors) appears to allow the target cells to survive the effects of toxic agents by interacting with cellular processes that are separate from genome repair. Split dose recovery experiments also indicate that genomic recovery occurs whether the factor is present or not. The factor may represent a new function for previously describe cytokine or may be itself be a newly discovered cellular agent.

These studies were supported by grants from the NJ Commission for Cancer Research and the UMDNJ Foundation (HZH) and the American Cancer Society (GJH).

O 209 **Glutathione S-Transferase (GST), a key anti-oxidant: new tools for analysis and modulation.** Lawrence M. Kauvar; Terrapin Technologies, Inc.; 750-H Gateway Blvd.; So. San Francisco, CA 94080

Glutathione S-Transferase (GST) is a family of abundant intra-cellular isozymes which inactivate electrophilic compounds by conjugating them to the abundant scavenger peptide glutathione (GSH). The primary source of toxic electrophiles in the cell is free radicals and peroxides leaking out of mitochondria. Possibly in response to inducers from surrounding necrotic cells, tumors often display elevated levels of one or more GST isozymes, presumably accounting for patient variability in response to alkylating agents used in chemotherapy.

Terrapin has prepared an extensive set of analogs of glutathione and used them to separate GST isozymes at high resolution under non-denaturing conditions, allowing better analysis than has previously been possible.

The same ligands have also been used to probe the active site of the isozymes. Several ligands of substantial specificity for one or another isozyme have been identified, with maximal potencies in the 1-10 micromolar range. Use of these ligands to inhibit GST in cells potentiates the cytotoxicity of an alkylating agent.

O 210 REGULATION OF ZOOID LIFESPAN IN BOTRYLLUS SCHLOSSERI IS DETERMINED BY A CLOCK MECHANISM THAT IS BUD-DEPENDENT, Robert J. Lauzon and Wen-Teh Chang, Department of Pediatrics, Albany Medical College, Albany, NY 12208

Botryllus schlosseri is an encrusting colonial ascidian of shallow waters and harbors throughout the world which embodies three developmentally-synchronized asexual generations of individuals. Every 5 days at 21°C, the older generation within a colony (zooids) dies over a 24 hour period, and is replaced by a new asexual generation. This cyclical death process known as **takeover** is characterized by three stages: (1) the shutdown of both siphons, which defines its onset; (2) a polarized contraction of zooids along their anteroposterior axis, and (3) the involution phase in which dying visceral tissues are engulfed by macrophages. Little is known about the mechanism(s) which regulate zooid regression, and whether lifespan is internally programmed or trigerred by a bud-dependent event. In order to determine the function of buds during takeover, buds of various developmental stages (secondary bud disc [A], skewing and closed vesicle [B-1, B-2], and organogenesis [C-1, C-2] stages) were removed using microsurgical techniques. The results can be summarized as follows: **(A)** in experiments where all buds were removed at stages B-1 through C-2, the onset of zooid death was identical to control isogenic clones. However, zooid contraction and involution rates were markedly altered in bud-deprived animals. Contraction was 2-3 fold less than in controls, and zooids never completely resorbed. On the other hand, when only half the number of buds were removed, the polarized contraction was only impaired in those zooids in which buds had been removed; **(B)** if all buds were removed at stage A, the onset of zooid death was delayed by 48-72 hours until new buds regenerated. However, removal of only half the number of buds at this stage restored the timing mechanism. Since heartbeat in the primary bud first begins during the B-1 stage of the asexual cycle, these findings strongly suggest that a bud-derived factor released throughout the colonial vasculature irreversibly determines zooid lifespan. Our results further indicate that buds confer polarity to the contraction process, and are essential for the removal and reabsorption of dying tissues through the vasculature.

O 211 IMMORTALIZATION PHENOTYPE DISSOCIATED FROM THE PRENEOPLASTIC PHENOTYPE IN MOUSE MAMMARY EPITHELIAL CELLS IN VIVO, Daniel Medina, Department of Cell Biology, Baylor College, of Medicine, Houston, TX 77030

Mouse mammary epithelial cells (MMEC) isolated from normal virgin BALB/c female mice and grown in cell culture for various lengths of time were injected into the mammary fat pads of syngenic mice. Of the ductal outgrowths which resulted from the injected MMEC, four gave rise to outgrowths which were serially transplanted beyond the lifetime of normal ductal outgrowths. The lifetime of normal ducts is 5 to 6 transplant generations. The four ductal outgrowth lines termed EL for "extended life" have been serially transplanted for 9, 11, 15 and 17 transplants generations. The outgrowths are predominately ductal in morphology, do not exhibit intraductal epitheliosis characteristic of ductal hyperplasias, are ovarian dependent for growth and are responsive to prolactin-mediated alveolar differentiation. Three of the EL lines, EL5, 7, and 11 have not produced any tumors spontaneously (0/64) and only one tumor after DMBA treatment (1/30). The fourth line, EL12, differs from the other three in the presence of a limited degree of alveolar differentiation. The EL12 line has not produced any spontaneous tumors (0/23) but is somewhat more responsive to DMBA (3/10). The four EL lines represent cell populations where the immortalized phenotype is dissociated from the hyperplastic phenotype which is characteristic of mouse mammary preneoplastic populations. The tumor suppressor gene, p53, is not overexpressed in the EL ductal outgrowths. As such, they may represent the earliest stage observable in the genesis of mouse mammary tumors and provide unique cell populations to examine molecular alterations associated with the property of immortality.

O 212 **EVIDENCE FOR A MATURATION-RELATED RESPONSE TO THE TERMINAL DIFFERENTIATION INDUCER CHOLERA TOXIN IN HUMAN MELANOCYTES**, E.E. Medrano, H-Y. Park*, R.E. Boissy, F. Yang, and J.J. Nordlund, Department of Dermatology, University of Cincinnati College of Medicine, Cincinnati, Ohio, and *Department of Dermatology, Boston University School of Medicine, Boston, MA.

Human melanocytes, whether derived from adult or neonatal skin, proliferate well in medium supplemented with phorbol esters (for >25 population doublings) before they undergo senescence (cessation of cell division). Pharmacological agents that raise intracellular cAMP induced early passage adult, but not neonatal, melanocytes to undergo terminal differentiation (characterized by morphological and biochemical changes). Cells became flat and enlarged. At the EM level we found numerous individual and compartmentalized melanosomes. There is a 5-6 fold increase in cAMP levels, in tyrosinase activity and in melanin content. There is also an increase in tyrosine kinase activity related to two proteins, $p38^{tyr}$ and $p40^{tyr}$. Other kinases, like protein kinase A, remained virtually unchanged in proliferating (neonatal) vs. terminally differentiated (adult) melanocytes. Altogether the changes suggest the induction of a terminally differentiated cell, although such phenotype has not yet been clearly defined. Interestingly, several of the human melanoma cell lines tested follow, upon CT treatment, the differentiation program observed in the adult melanocytes. Normal melanocyte cultures that are just a few passages away from senescence are strikingly more sensitive to cAMP elevating agents than are early passage cultures. Senescent and terminally differentiated melanocytes obviously share the feature of irreversible growth arrest. However, the senescent cells do not show the large increase in melanin characteristic of terminally differentiated cells. Thus in human melanocytes senescence and terminal differentiation are not identical phenotypes, although they may share early regulatory steps.

O 213 "TRANSFORMATION" OF HUMAN DIPLOID FIBROBLASTS FOLLOW STABLE TRANSFECTION OF HUMAN CDC_2 AND CYCLIN A GENES, T. Norwood*, M. Ohsubo†, J. Roberts†, A. Saulewicz*, *University of Washington, †Fred Hutchinson Cancer Research Center

The genes for human cyclins A, B and E and the cyclin dependent kinases cdc_2 and cdk2 were transfected, via a retroviral vector with a neo resistant marker, into separate late passage (85-90% of lifespan expended) human foreskin cultures derived from a normal newborn donor. Cultures resistant to G-418 were recovered following the viral infection and propagated to senescence. Presence of increased levels of these transfected gene products were demonstrated by Western blot analysis in all cultures consistent with constitutive synthesis directed by the LTR promoters in the viral vector. However, in the cyclin A culture only a modest increase over non-infected controls was seen. The cultures transfected with cyclins B and E, cdk2 and a retrovirus with no insert entered the post-mitotic, senescent state at approximately 65 population doubling with no apparent alterations of proliferative potential. In constrast, multiple foci of proliferating cells appeared in non-senescent cultures that had been transfected with cyclin A and cdc_2. The clones were pooled and propagated by weekly passaging. As of submission of this abstract, the cdc_2 and cyclin A cultures have achieved approximately 90 population doublings. Both cultures display a relatively low growth rate, in the range of 1-2 population doublings per week. The cells in the "transformed" cultures displayed markedly differing morphologies. The cdc_2 infected cells are spindle shaped and display size heterogeneity and extensive micronucleation. In constrast, the cyclin A infected cells are more polygonal with less size variation and minimal nuclear aberrations. Chromosome studies revealed multiple clonal aberrations; structural aberrations involving the long arm of a chromosome 11 homologue and the short arm of a 17 homologue were documented in both cdc_2 and cyclin A cultures. In addition, monosomy 6 was observed in all cells in both cultures; other clonal aberrations distinctive to each culture were detected. No chromosome aberrations were seen in virus infected cultures harvested prior to the transformation event. Careful analysis of chromosome morphology has revealed a similar array of heteromorphisms in the parental and transformed cultures.

O 214 INDUCTION OF CELLULAR SENESCENCE IN HUMAN CERVICAL TUMOR CELL LINES BY p53.

Vicente Notario, John F. Loome, Joseph A. DiPaolo*, Anatoly Dritschilo and Juan A. Velasco, Department of Radiation Medicine, Georgetown University Medical Center, Washington, DC, and *Laboratory of Biology, N.C.I., N.I.H., Bethesda, MD.

Overexpression of the wild-type p53 tumor suppressor gene inhibits the growth of a variety of normal and tumor cell lines. This inhibitory effect seems to be mediated through mechanisms involving programmed cell death, particularly in cell lines which do not express p53. Preliminary studies have shown that overexpression of wild-type p53 in human cervical tumor cell lines slows growth and down-modulates the tumorigenic phenotype. Retroviral vectors expressing p53 sense transcripts [pZip-Neo-SV(X)] have been made and used to lipofect cervical tumor cells (C-4I, ME-180, C33-A, and SiHa) which differ in level and/or type (wild-type vs. mutant) of p53 endogenous expression, and the type (16, 18 or none) of papillomavirus they contain. Regardless of the HPV type, transfection with p53 sense constructs resulted in drastically lowered transfection efficiencies (as total number of neo^R colonies relative to control cultures), and poor colony growth rate and viability. Particularly in the case of ME-180, sense-transfected cells showed significant morphologic alterations consistent with the involvement of apoptotic pathways. At various times after gene transfer, the cell division cycle of individual clones was arrested, and membrane-bound figures and nuclear condensation became apparent under the microscope. In situ hybridization, and recombinant vectors for the conditional expression of p53, and antisense DNA and RNA approaches are currently in use to understand the regulation of p53 in human cervical tumor cells.

O 215 **AGE-ASSOCIATED PHOTOCHEMICAL CROSSLINKING OF RAT EYE LENS CRYSTALLINS.**

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Eye lens proteins are not turned over and are extensively exposed to light radiation, resulting in photo-induced damage that contributes to lens opacification and may be involved in cataract formation. Characterization of the molecular alterations in these proteins are thus of great significance. Spectroscopic studies of rat eye lens crystallins isolated from young (2 month) and old (26 month) animals show a modification of some tryptophan residues with age. These spectroscopic changes occur in parallel with the age related formation of insoluble crystallin aggregates involving non-disulfide covalent crosslinks. Time-resolved fluorescence analysis of whole rat lenses and of chromatographed lens crystallins show that the majority of old lens crystallins with a red shifted absorbance and a red shifted fluorescence component (420nm peak emission) are retained within the insoluble phase during lens protein extraction. The red shifted fluorescence may reflect a covalent modification of tryptophan residues which supports earlier suggestions that tryptophan is involved in the non-disulfide crosslinking of crystallins. While non-disulfide crosslinks are the cause of crystallin aggregation, the exposure of one or more tryptophan residues to solvent as evidenced by iodide quenching studies reflects conformational changes of crystallins following the formation of intra-molecular disulfide linkages. Our time resolved fluorescence measurements show the reduction of a short 700 picosecond tryptophan fluorescence component in old whole rat lens suggesting that a highly quenched tryptophan is either destroyed or forced into another less quenching environment. Since extensive non-disulfide crosslinking occurs only after the formation of the intramolecular disulfide crosslinks, this suggests the possibility that the newly exposed tryptophan is the most susceptibile to covalent crosslinking.

Exclusion gel chromatography profiles of the soluble components of lens crystallins indicate that γ-crystallin (expressed in young animals only) is almost entirely removed from the soluble protein phase. γ-crystallins isolated from young rat lens (2 months) already have abnormal fluorescence characteristics indicative of a covalent modification involving either a tryptophan photoproduct, bityrosine or glycosylation.

O 216 ROLE OF INTERLEUKIN 1B IN THE EXPRESSION OF APP IN A HUMAN ASTROGLIOMA CELL LINE

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The main hallmark of Alzheimer's disease (AD) is the presence in the brain of dense core plaques which contain large amounts of β-amyloid peptide(β-A4). β-A4 is thought to be derived from a precursor protein called APP. The mechanism by which β-A4 is produced is not known but several lines of evidence suggest that neurodegeneration might be related to the toxicity of the β-A4. Any increase in the expression of APP could lead to a higher probability of β-A4 formation. An inflammatory response clearly occurs in AD brain since high levels of interleukin 1 (IL-1) have been reported as well as the presence of acute phase proteins.

We have used the human astroglioma cell line (U373-MG) as an in vitro system to look at the production of IL-1β after stimulation with endotoxin, and at the regulation of APP expression after IL-1β or endotoxin stimulation. 10μM LPS stimulated the production of IL-1β as revealed by ELISA with a peak occuring at 6 h. The interleukin 1β converting enzyme (ICE) was also found to be overexpressed after LPS stimulation, suggesting that the mature form of IL-1β was produced. Quantitative PCR was used to examine the expression of the different forms of APP after IL-1β action.

O 217 NEUROTOXICITY OF RIBONUCLEASES,

Richard J. Youle, Dianne Newton, Wojciech Ardelt, Stanley Mikulski, and Susanna M. Rybak, Surgical Neurology Branch, NINDS, NIH

Purkinje cell loss occurs with aging. Purkinje cell toxicity is also one of the characteristic features of the Gordon Phenomenon, a syndrome manifested by ataxia, muscular rigidity, paralysis, and tremor that may lead to death. Two members of the RNase superfamily, EDN (eosinophil derived-neurotoxin) and ECP (eosinophil cationic protein) cause the Gordon phenomenon when injected intraventricularly into guinea pigs or rabbits. Another member of the RNase superfamily, onconase, isolated from *Rana pipiens* oocytes and early embryos will also cause the Gordon phenomenon when injected into the cerebrospinal fluid of guinea pigs at a dose similar to that of EDN (LD50). The neurotoxic activity is dependent on RNase enzyme activity. When onconase is modified to varying extents by iodoacetic acid leading to a 70% and 98% decrease in RNase activity, the ability of the modified onconase to produce the Gordon phenomenon closely parallels the remaining RNase activity. In contrast, bovine pancreatic RNase A injected intraventricularly at a dose 5000 times greater than the LD50 dose of EDN or onconase does not cause the Gordon phenomenon. Human ribonucleases cause neuronal cell loss *in vivo*. Although RNase activity appears essential for neurotoxicity, other determinants cause wide differences in the potency of homologous ribonucleases.

Genes and Gene Regulation

O 300 PHOSPHORYLATION OF THE p53 TUMOR SUPPRESSOR PROTEIN BY THE DNA-ACTIVATED PROTEIN KINASE, Carl W. Anderson[1], Susan P. Lees-Miller[1], David Lin[2], W. Edward Mercer[2], Kazuyasu Sakaguchi[3], Michele Fiscella[3], Stephen Ullrich[3], and Ettore Appella[3]; [1]Biology Department, Brookhaven National Laboratory, Upton, NY 11973; [2]Jefferson Cancer Institute, Thomas Jefferson University, Philadelphia, PA 19107; [3]Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD 20892.

DNA-PK is a nuclear serine/threonine protein kinase that requires linear, double-stranded DNA for activity. DNA-PK activity is tightly associated with a nuclear 350-kDa polypeptide, and recent evidence suggests the Ku autoantigen also may be required for DNA-PK activity. DNA-PK phosphorylates several nuclear DNA-binding proteins including the SV40 large tumor antigen, RPA, Oct-1, Fos, SRF, the CTD of RNA polymerase II, and the p53 tumor suppressor protein. These findings suggest DNA-PK may regulate nuclear functions, perhaps in response to DNA damage.

We have identified two sites in human and mouse p53 that can be phosphorylated by DNA-PK. Using synthetic peptides, we show that recognition of these sites depends on the presence of an adjacent glutamine and is inhibited by nearby arginines and lysines. *In vitro*, p53 phosphorylation is strongly enhance when it and DNA-PK bind to the same DNA fragment. Changing serine 15 of human p53 to alanine altered p53 expression and its ability to inhibit cell cycle progression. These findings suggest a model for the activation of p53's ability to suppress tumor cell growth.

A rapid and highly specific assay for DNA-PK activity has been developed; DNA-PK is moderately abundant in all primate cells examined, and the activity is reduced or absent in rodent cells. Partial cDNA clones for the human 350-kDa DNA-PK polypeptide have been obtained; the gene for this polypeptide has been located on human chromosome 8 by *in situ* hybridization.

O 301 DNA BINDING ACTIVITY DECREASES IN AGING HUMAN DIPLOID FIBROBLASTS, Peter Atadja, Claude Veillette, Karl Riabowol, SACRC, Department of Medical Biochemistry, University of Calgary, Calgary, AB, Canada T2N 4N1

Fos, the product of the proto-oncogene c-*fos*, is a protein that is necessary for proliferation of human diploid fibroblasts in culture. Fos is a major component of the AP1 transcription factor complex and previous data from our laboratory indicate that expression of the Fos protein is decreased several fold in aging and senescent cells. Furthermore, the reduced amount of Fos that is expressed does not appear to be active as measured by ability to bind DNA.

Since other labs have shown that decreased transcription of the c-*fos* gene is one of the mechanisms by which expression of Fos protein is reduced, we have begun a comparative study of factors binding upstream of the c-*fos* gene in early and late passage HS-68 human fibroblasts. Synthetic DNA primers were used to generate overlapping 100 bp oligonucleotides by PCR that correspond to regions upstream of the gene known to be relevant for transcription. When used as probes in DNA mobility-shift assays, a striking decrease in the formation of DNA-protein complexes was observed for all four probes with extracts from the nuclei of old, as compared to young cells. Additionally, proteins extracted from the nuclei of aged cells bound shorter probes corresponding to the human c-*fos* SRE/AP1, CRE, AP1, E2F and RCE motifs and the actin-SRE at much lower levels than nuclear proteins from young cells. Such results were highly reproducible from different protein preparations and were done in the presence of several protease inhibitors and reducing agents making it unlikely that decreased binding resulted from non-specific proteolysis or oxidation during protein extraction. Furthermore, total nuclear proteins appear quantitatively and qualitatively similar at the levels of ^{35}S-methionine and silver stain profiles. These methods, however, would not be expected to detect variations in proteins expressed at very low levels. Experiments are currently underway to enrich for binding proteins to compare their expression and modification profiles in young and old cells. Preliminary data indicate that specific changes in kinase activities occur as cells age *in vitro* which might explain, in part, loss of DNA binding activity with increased passage level.

O 302 SERUM GLYCOPROTEIN HORMONES AND THEIR FREE α-SUBUNIT IN A HEALTHY ELDERLY POPULATION SELECTED ACCORDING TO THE SENIEUR PROTOCOL, Peter Berger, Stephan Madersbacher, Thomas Stulnig, Lukas Huber and Georg Wick, Institute for Biomedical Aging Research and Institute for General and Experimental Pathology, A-6020 Innsbruck, Austria

In the process of aging striking endocrinological alterations occur at all levels of the hypothalamic-pituitary-gonadal axis. Reliable analyses of such changes require well characterized study populations, e.g., those defined by the SENIEUR protocol, which originally was designed for immunogerontological studies by the European Economic Community Concerted Action Programme on Aging (EURAGE). Chorionic gonadotropin (CG), free glycoprotein hormone α-subunit, luteinizing hormone (LH) and follicle stimulating hormone (FSH) serum levels were analyzed in individuals considering themselves as healthy but either meeting SENIEUR criteria or not (NON-SENIEUR). Analyses were performed by ultrasensitive (2 pg/ml) highly specific time-resolved immunofluorometric assays (IFMAs) using our own panel of monoclonal antibodies. Values were correlated to age and SENIEUR status. Surprisingly, significant increases ($p < 0,01$) of the pregnancy hormone and tumormarker CG and free α-subunit but also, as expected, of LH and FSH was observed between old and young females (n=37); In males (n=38) a significant increase with age of LH and FSH but not of CG and free α ($p < 0,05$) was seen. Two-factor ANOVA analyses of variance revealed no impact of the SENIEUR status, neither on the hormone levels nor on age-dependent differences in both sexes.

O 303 AN AGE DEPENDENT TRANS-ACTING FACTOR(S) REGULATES A HUMAN FACTOR IX GENE PROMOTOR IN A TRANSGENIC MOUSE LINE. E.J. Boland, M. Odom, C.A. Walter, D.C. Herbert, F.J. Weaker, B.H. Bowman, G.G. Brownlee and P. Jagadeeswaran. Department of Cellular and Structural Biology, UTHCS at San Antonio, San Antonio, TX. 78284-7762.

Expression of the X-linked Factor IX (F.IX) gene by hepatocytes is essential for normal blood coagulation. A decrease in F.IX synthesis can result in the bleeding disorder, hemophilia B. One variant, hemophilia B_{Leyden}, displays a distinct age-dependent phenotype in which F.IX synthesis is absent during childhood, however; beginning at puberty and continuing thereafter, F.IX synthesis increases and normal clotting activity is observed. An increase in the level of F.IX synthesis, which occurs in the elderly, can result in spontaneous emboli formation often leading to stroke. These data are indicative of the presence of tissue specific factor(s) which regulate the F.IX promotor in an age-dependent manner. In order to examine the molecular basis of these age-dependent phenotypes *in vivo*, we have generated two lines of transgenic mice; one bears a chimeric gene containing 189 bp of the 5' regulatory region of the FIX gene fused to the chloramphenicol acetyl transferase (CAT) reporter gene (-189(N)) and the second (-189(+13)) differs only by containing an A->G point mutation at the +13 position which has been implicated in hemophilia B_{Leyden}. Liver tissue extracts were examined for CAT activity at 2 and 8 months postnatal age (murine sexual maturation occurs around the second month). At two months, both male and female -189(N) mice displayed detectable CAT activity. At 8 months, the -189(N) mice display increased CAT activity while in the -189(+13) mice, males display CAT activity whereas females do not. Mobility shift assays have identified an age-dependent factor present in the 8 month old mice which is not present at 2 months. These data provide the first *in vivo* evidence that (1) an age-dependent trans-acting factor(s) is involved in the expression of the F.IX gene and (2) androgens are involved in the regulation of the F.IX gene. They also support the use of the transgenic model for future studies of molecular factors important in aging and F.IX gene regulation.

O 304 STUDIES OF THE AGING PROCESS IN TRANSGENIC MICE, Barbara H. Bowman, Gwen Adrian, Damon Herbert, Chris Walter, Frank Weaker, and Erle Adrian, Department of Cellular & Structural Biology, The University of Texas Health Science Center, San Antonio, USA

Transgenic animals provide the means of correlating gene expression during aging with characterized sequences of chromosomal DNA. A human chimeric gene encoding human serum transferrin has been chosen to study the aging transgenic mouse model. The usefulness of the system lies in its capacity to demonstrate expression of the transgene in every appropriate cell type during each stage of mammalian development, from early embryogenesis through the aging process. Expression of the human chimeric transferrin gene (TF) was similar to the tissue patterns of mouse and human transferrin. Expression of the human chimeric transferrin gene diminished 40% in livers of transgenic mice between 18 and 26 months of age. The age-related decrease is at the transcription level and involves at least one identified DNA region of the human TF gene. Transferrin levels and serum iron levels in aging humans also diminish, as observed from measurements of total iron binding capacity and percent iron saturation in sera from 701 individuals ranging from 0 to 99 years of age. This may present a basis of dysregulation of iron during aging. In contrast, in transgenic mice and nontransgenic mice, the mouse endogenous plasma transferrin and endogenous transferrin mRNA increase significantly during aging. Although the regions of the human transferrin and mouse transferrin genes are homologous, DNA sequence diversities do exist which could account for the different responses of the two species to aging. Valuable hints about the DNA sequences that respond to the aging process have been gained from these studies. The correlation of specific DNA sequences and changes in age-related gene expression will accelerate an understanding of the physiological and environmental factors that contribute to the aging process.

O 305 MULTICOMPONENT REGULATION OF ANDROGEN SENSITIVITY DURING MATURATION AND AGING, B. Chatterjee, M.H. Jung, S. Her, M. Slomczynska and C.S. Song, Dept. Cellular & Structural Biology, The Univ. of Texas Health Science Center & Audie L. Murphy VA Hospital, San Antonio, TX 78284

During its life span, the rat liver exhibits a triphasic pattern of androgen sensitivity. The hepatic response to androgens changes from hormone insensitivity in the prepubertal life to androgen responsiveness in the young-adult animal, and a subsequent gradual decline and ultimate loss of the hormonal response during aging. Appearance of androgen sensitivity at puberty is correlated with hepatic induction of the mRNAs for androgen receptor (AR) and estrogen sulfotransferase (EST), and repression of the mRNAs for dehydroepiandrosterone sulfotransferase (DST). Estrogens are antagonistic to the AR, and sulfated steroids are known to be receptor-inactive. Thus reciprocal regulations of EST and DST potentiate androgen receptor action in the liver of postpubertal adult rats. Aging is associated with a decline and eventual loss in the expression of AR and EST, whereas DST expression increases steadily. The androgen modulates hepatic expression of both EST and DST, acting as a positive regulator for the former and a negative regulator for the latter. The loss of AR mRNAs during aging can be prevented in food-restricted rats, thus resulting a reversal in the regulation of EST and DST in senescent rats. We have discovered several transcription factors that play important roles in the regulated expression of the AR gene. Activities for some of these transacting factors change during aging in parallel with the temporally altered regulation of the AR gene. Further characterization of these transcription factors will facilitate our understanding of the molecular basis for changes in hepatic androgen sensitivity during maturation and aging.

O 306 THE 5' UPSTREAM REGULATORY REGION OF THE RAT AMYLOID PRECURSOR PROTEIN GENE

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The 5' upstream regulatory region of the rat amyloid precursor protein (APP) gene has been cloned and sequenced. It is very GC-rich and highly homologous to the corresponding nucleotide sequence of the mouse and human APP genes. The rat upstream region contains putative regulatory elements both 5' and 3' to the probable transcriptional start. There are 3 candidate SP1 binding sites, several possible AP1, AP2, and AP4 binding sites, and several GC-rich boxes of the form GGGYGCRG. The rat SP1 sites are more closely related to the consensus binding site than the similar sites in the human. However, one of the human candidate AP2 sites, as well as most of the human GC-rich boxes, are entirely missing in the rat. Therefore, while all three species may contain several regulatory elements, the identities and strengths of these elements may vary significantly from species to species. The observed similarities and differences between the 5' upstream regulatory regions of the rat, mouse and human APP genes may be functionally related to the appearance of senile amyloid plaques in older humans and Alzheimer's patients but not in older or memory-impaired rodents.

Additional structural features of the rat, mouse, and human sequences will be presented, along with data regarding the ability of the rat 5' upstream sequence to regulate expression of the APP gene.

O 307 Molecular Characterization of Y13, a Gene Preferentially Expressed in Young Cells of **Saccharomyces cerevisiae.**

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The yeast **Saccharomyces cerevisiae** possesses a defined life span. It is defined by the replicative capacity of the cell. This phenomenon of a defined life span or "aging", may be controlled or regulated by the interaction of a number of genes. Several genes have been isolated by differential hybridization, which are preferentially expressed during the life span of the yeast cell. One of these genes, Y13 is preferentially expressed in young cells and is being characterized for its role in yeast aging. Mapping of the 5' end of the 2.4 Kb transcript, with both Nuclease S_1 and primer extension, demonstrated a single transcription start site. Y13 is a single copy gene with sequence similarity, by Southern blot analysis of genomic DNA, to human and other mammalian species. Analysis of the DNA sequence revealed two possible transmembrane helicies and a predicted PEST region, located near the N-terminus of the protein. Therefore, this gene may encode a membrane protein whose stability may be determined by the PEST region. We are examining the effect that Y13 disruption and overexpression may have on the mean and maximum life span of the yeast cell.

O 308 FUNCTIONAL CHARACTERIZATION OF A LONGEVITY ASSURANCE GENE (*LAG1*) IN YEAST, Noel P. D'mello, David S. Franklin, Adele M. Childress, Chutaphant Pinswasdi, Shubha P. Kale, Neil E. Jeansonne, and S. Michal Jazwinski, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112

The life span of the yeast *Saccharomyces cerevisiae* is characterized by the number of divisions the cell undergoes. During its life span the cell produces a finite number of daughter cells. Several genes have been identified that are expressed preferentially as the cell ages. We have characterized one of these genes, *LAG1* (longevity assurance gene), that is expressed primarily in young yeast cells.

Disruption and deletion of the gene in haploid cells showed that it is non-essential for vegetative growth and division. Disruption of the gene which was performed at the 3' end causes a significant decrease in the life span of the cells. Deletion of the 5' end of the gene has no effect on life span. However, elimination of *LAG1* expression results in an increase in the mean and maximum life span. Overexpressing the gene in young cells appears to decrease the replicative capacity, whereas overexpression in older cells results in an extension of the life span. These conclusions are drawn from experiments utilizing two different inducible promoters. Phenotypic analysis of the cells with the deleted *LAG1* gene showed no differences when compared with the parent strain. However, cells in which the gene was disrupted were unable to grow on non-fermentable carbon sources. They were able to grow faster on caffeine, when compared to the parent strain. We propose a formal model to elucidate the function of the gene in the process of aging. These results suggest that the gene is involved in the aging process of yeast perhaps through the *RAS*-cAMP pathway.

O 309 **ISOLATION OF GENES THAT ARE DIFFERENTIALLY EXPRESSED DURING AGING IN *C. ELEGANS*, .**

Thomas J. Fabian[1,2] and Thomas E. Johnson[1], Department of MCD Biology and Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80303-0447

To isolate cDNA clones for genes which are differentially expressed during aging in *C. elegans*, we differentially screened duplicate filter lifts from a nematode cDNA library with radiolabelled cDNA probes synthesized from mRNA isolated from young adult and aged adult nematodes. Of 2×10^4 plaques screened, approximately 3000 were detected with the young and/or the aged adult probe. Of the plaques detected, about 60 - 70 appeared to give a differential signal. Thirteen distinct clones (designated "Y") gave a greater signal with the young adult cDNA probe and 9 distinct clones (designated "S") yielded a greater signal with the aged adult cDNA probe. In addition, three clones (designated "NC", no change) were randomly chosen from the majority which did not give a differential signal. To verify the age-dependent expression of these clones, additional nematode cultures were grown, and the age-specific RNAs isolated from these cultures were used for Northern and quantitative dot blot analyses. The results obtained to date indicate that several of the Y clones (and, in particular, clones Y15 and Y37) showed replicable decreases in mRNA abundance with age. Only one of the S clones (S3), however, appears to exhibit a substantial, replicable increase during the adult life span. We have also examined expression of several clones in a strain carrying an allele of *age-1* that results in a lengthened life span. With the possible exception of the mRNA abundance of *sod-1* (the gene coding for the Cu/Zn form of superoxide dismutase), no obvious differences in age-dependent mRNA abundances between the long-lived *age-1* strain and strains with wild type life spans have been observed.

O 310 A YEAST HOMOLOG OF THE RAT PROHIBITIN ANTIPROLIFERATIVE GENE IS DIFFERENTIALLY EXPRESSED AND DETERMINES LONGEVITY IN *Saccharomyces cerevisieae*, David S. Franklin and S. Michal Jazwinski, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112

The rat prohibitin (PHB) gene has been previously described. This gene encodes an antiproliferation protein that is expressed in quiescent rat liver cells rather than regenerating liver cells. Microinjection of synthetic rat PHB mRNA into normal fibroblasts or HeLa cells can block entry into S phase. Microinjection of an antisense oligonucleotide for rat PHB mRNA can stimulate entry of rat liver cells into S phase. Recently, a yeast homolog of the rat prohibitin gene has been isolated in *Saccharomyces cerevisieae* (D. Stewart, Personal Communication). We have examined the role of the yeast PHB homolog in determining longevity in yeast cells. The yeast PHB gene produces mRNA about 0.95 Kb in size. The gene is preferentially expressed in young yeast cells, and its expression decreases as the cell ages. Deletion of PHB in haploid cells is nonlethal and can extend the yeast lifespan, both mean and maximum, by approximately 30%. Overexpression of PHB decreased the mean lifespan by 20%. The yeast PHB deletion and overexpression results parallel the effects obtained by the microinjection of an antisense oligonucleotide for rat PHB mRNA and microinjection of synthetic rat PHB mRNA experiments mentioned above. Though functionally similar, the expression of the yeast PHB gene seems contrary to its rat homolog. In rat liver cells, PHB is expressed in quiescent or nondividing cells. However, young yeast cells, which preferentially express PHB, are equivalent to actively dividing cells. The yeast PHB gene is a novel longevity assurance gene capable of setting limits on the replicative age of yeast cells. Deletion of the PHB gene might serve to negate these limits and allow additional divisions. Further study of this gene might give insight in to how proliferation is regulated in yeast cells and what might cause senescence.

O 311 ALPHA1-ANTICHYMOTRYPSIN INHIBITS AND IS COLOCALIZED WITH 'PROHORMONE THIOL PROTEASE' INVOLVED IN PROENKEPHALIN PROCESSING: IMPLICATIONS FOR ALZHEIMER'S DISEASE. Vivian Y.H. Hook and Rosalyn T. Purviance, Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda,MD 20814

Alpha1-antichymotrypsin (ACT) is developmentally regulated in brain and is abundant in amyloid deposits of Alzheimer's Disease. Identification of target protease(s) inhibited by ACT in the nervous system is important for understanding ACT's function in normal brain and in Alzheimer's Disease.

Neuronal chromaffin cells of adrenal medulla have been extensively used as a model system to study neurotransmitter synthesizing enzymes including proteases that convert protein precursors to active peptide neurotransmitters. We have identified a novel 'prohormone thiol protease' (PTP) as the major proteolytic activity responsible for converting proenkephalin to (Met)enkephalin and related neuropeptides in adrenal medullary secretory vesicles (known as chromaffin granules),.

Most importantly, we find that an endogenous ACT-like protein inhibits the 'prohormone thiol protease' (PTP). ACT-like immunoreactivity is colocalized with PTP in secretory vesicles. Purified endogenous bovine ACT-like protein effectively inhibits PTP in the nM range, and forms a SDS-stable complex with PTP. These findings suggest that a role for brain ACT in regulating proteolysis of neuropeptide precursors should be investigated.

O 312 ALTERATIONS OF HUMAN CHROMOSOME 6 IN IMMORTALIZATION AND CELLULAR SENESCENCE, Karen Hubbard-Smith, Arbansjit K. Sandhu, Gursurinder P. Kaur, Raghbir S. Athwal, and Harvey L. Ozer, Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ 07103

Human diploid fibroblast (HDF) can escape cellular senescence by SV40 transformation. Transformed HDF have an extended but limited lifespan; immortal transformants can be isolated at low frequencies. We have generated a series of genetically-matched sets of pre-immortal and immortal SV40 transformants to identify alterations in the cellular genome which may play a role in immortalization. Chromosomal aberrations involving chromosome 6q occur in SV40 immortal cell lines. Cytogenetic, Southern, and PCR analyses demonstrated a non-random loss at and/or distal to 6q21. PCR analyses of a microsatellite polymorphism at 6q22 show a loss of heterozygosity (LOH) of this locus in immortal cells. Two independent series of SV40 transformants were found to have a complete loss of the long arm of chromosome 6. Another independently derived immortal transformant had multiple copies of chromosome 6, but also showed LOH for 6q22. To establish that the immortal phenotype was directly associated with loss of 6q, a normal human chromosome 6 was introduced into immortal cell lines by microcell-mediated chromosomal transfer. Microcell hybrids exhibited growth suppression and a senescent-like morphology. Our studies have identified a putative growth suppressor gene on chromosome 6, which may have an important role in cellular senescence and immortalization.

O 313 AN AGE-RELATED DECLINE IN THE OSTEOBLAST PROGENITOR POOL IN MARROW: INDICATIONS OF A ROLE FOR TGF-B, Arnold Kahn and Dan Gazit, University of California, San Francisco, CA and the Hebrew University-Hadassah Medical Center, Jerusalem, Israel.

In the present study, we have tested the hypothesis that the lowered bone forming activity seen in older animals and man is the result of a diminution in the quantity and quality of the osteoblast progenitor pool and have initiated assesment of the notion that this is the result of a decrease in the level/activity of TGF-b. Bone marrow was collected from BALB/c mice at 4 and 20-24 months of age and cultured, *in vitro*, to determine the content of putative osteoblast progenitors. Samples of bone tissue were also collected for histological analysis and to determine the content of TGF-b in the bone matrix. Finally, putative progenitor cells were evaluated for their expression of the TGF-b receptor and for their responsiveness to TGF-b. Our observations confirm the loss of bone in old animals and demonstrate that matrix from old mice contains ~50% less TGF-b, indicative, we believe, of reduced TGF-b synthesis with age. More critically, we find an ~65% decline in old mice in the number of fibroblast colony forming cells (CFU-F) with apparent osteoblastic potential (alkaline phosphatase activity). These cells, however, express higher levels of TGF-b receptor (perhaps reflecting up-regulation in response to lower levels of TGF-b *in situ*) and appear to be more responsive to added TGF-b. These data support the hypothesis of an age-related decline in the marrow osteoblast progenitor pool and are consistent the notion that such a diminution may be linked to changes in the synthesis/availability of TGF-b.

O 314 SIMILAR ROLES OF RAS1 AND RAS2 IN YEAST GROWTH BUT POSSIBLE ALTERNATE FUNCTIONS IN AGING, Shubha P. Kale , Jiayan Sun and S. M., Jazwinski, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112

In *Saccharomyces cerevisiae*, *RAS*1 and *RAS*2 genes encode homologous proteins with a 180 amino acid domain highly conserved with mammalian ras genes. Both *RAS*1 and *RAS*2 genes in yeast promote vegetative growth. A disruption of either of the genes alone has no effect. However, a disruption of both is lethal in haploid cells.

One of the functions of *RAS*2, in yeast, is to modulate the cAMP levels. We have demonstrated that *RAS*2 has an additional function; it is involved in determining longevity. It functions as a longevity assurance gene by extending the mean and the maximum life span by 30%, when overexpressed. To examine whether *RAS*1 plays a similar role, it was overexpressed in haploid yeast cells under the control of *GAL*10 promoter. Micromanipulation studies showed no significant effect on the mean or maximum life span. However, Northern blot analysis demonstrated that the expression of this gene decreased during the yeast life span.

These results suggest that every gene that is differentially expressed during the life span may not directly affect yeast longevity. *RAS*1 expression may decrease in old yeast cells either as a consequence of aging, or it may affect aging in an indirect manner. Thus, *RAS*1 may have a different function than *RAS*2 in yeast senescence.

O 315 INCREASED LIFE SPAN EXHIBITED BY TWO NEW MUTATIONS IN *C. ELEGANS*, Pamela L. Larsen, Division of Biological Sciences, University of Missouri, Columbia, MO 65211

A hypothesis relating damage accumulation to aging, predicts that increased longevity is associated with increased resistance to types of damage relevant to aging. I have previously demonstrated that *age-1*, which increases life span, increases resistance to oxidative stress. The *C. elegans* strain, CB4512, was a candidate for a heat shock resistant strain because it was isolated from a desert canyon. CB4512 also has a slow growth phenotype that was shown to be due to one gene, *gro-1* (J. Hodgkin, personal communication). CB4633 *gro-1(e2400)* and CB4512 both develop at approximately one half the rate of wild-type. They produce fewer progeny and display heat shock resistance. They have increased mean and maximal adult life spans over that of wild-type.

A mutation in another gene, *gro-2*, develops at a rate similar to CB4633. CB4570 *lev-1(x577) gro-2(e2442)* has an increased maximal adult life span, however, its mean life span is not significantly different than its parent *(lev-1)*. There is no reason to assume that all mutations which cause slow growth would increase life span. In fact, many slow growing strains are sickly and have decreased life spans.

The mutation in the *gro-1* locus confers heat shock resistance, impaired reproductive capacity, extended larval period, and increased life span. The simultaneous occurrence of these phenotypes is consistent with the theory on aging in which senescence occurs due to the lack of selective pressure which would maintain the organism after it has successfully reproduced. Thus, evolution would select genes that improve the success of reproduction and accept subsequent detrimental effects. In general, the pleiotropic effects of slowed development and small brood size should be selected against. However, the heat shock resistance might be beneficial in desert environments. An alternative explanation for the increased life span is that the entire life cycle is slowed by the *gro* mutations, i. e. predominantly nonstochastic mechanisms determine the observed life span. If the observed changes in *gro-1* and *gro-2* early in life are directly related to the increased life span, then this may provide insight into possible causes of senescence.

O 316 ISOLATION AND PURIFICATION OF A GROWTH INHIBITOR FROM NORMAL HUMAN FIBROBLASTS, A. Macieira-Coelho, A. Söderberg and A. Wasteson, Department of Cell Biology, Faculty of Health Sciences, 581 85 Linköping, Sweden.
Indirect evidence for the presence of a growth inhibitor in normal human fibroblasts has been obtained previously; the inhibitory activity has been found associated with crude cell extracts but the molecule responsible for the growth inhibition has never been isolated. We have isolated a glycopeptide from human fibroblast cultures, that decreases DNA synthesis when added simultaneously with a growth stimulus to resting cell cultures. It was separated by ion exchange and lectin affinity chromatography and by molecular mass (SDS-PAGE). Growth factors induce a decreased synthesis of the inhibitor at the early stages of the G1 period, then the synthesis of the inhibitor remains stable during S, and finally during the G2 period its synthesis reverts back to resting stage levels. The relationship of the kinetics of the glycopeptide with the cell division cycle, and its molecular weight, are different from what has been described so far for other growth-regulators. The decreased synthesis of this inhibitor, induced by growth factors, seems to be one of the requirements for the initiation of the division cycle by human fibroblasts. This response to growth factors was stable during the life span of the fibroblast population and became impaired in cells at the end of their replicative potential.

O 317 Characterization of a 33 kD form of APP-C100 from Stably Transfected PC12 Cells and Transgenic Mice

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We have generated stably transfected PC12 cells harboring various portions of the human amyloid precursor protein (APP). Notably, C100, 751 and 695 forms of APP have been expressed in these PC12 cells. In parallel, we are studying transgenic mice that express the C100 portion of human APP specifically in brain tissue (Sandhu et al, *J. Biol. Chem.*, 266, 21331-34, 1991). Using a polyclonal antibody, TC2, made against a peptide of APP residues 644-676, we have observed increased levels of a 33 kD protein on Western blots of lysates from C100 mice and PC12 cells compared to wild type cells or mice. Additionally, 751 but not 695 transfected PC12 cells display increased levels of the 33 kD protein. TC2 Binding to the 33 kD protein is specific and is eliminated by pretreating antibody with C2 peptide. Purification and protein sequence analysis reveal that the 33 kD protein is derived from our human transgene in both cells and mice. The 33 kD protein does not result from read through translation of the transgene nor is it glycosylated or phosphorylated. The nature of this potentially amyloidogenic form of APP will be discussed. Also, distribution of the 33 kD protein in Alzheimer's disease brain tissue will be investigated.

O 318 HOW AGING COMPROMISES POLYMERIC IMMUNOGLOBULIN RECEPTOR FUNCTION. Douglas L. Schmucker[1-3] and Christopher K. Daniels[4,5], Cell Biology & Aging Section, DVA Medical Center[1], the Liver Center[2] and Department of Anatomy[3] University of California, San Francisco, CA 94121; Clinical Pharmacology & Gerontology Research Unit, DVA Medical Center, Boise, ID 83702[4]; Department of Pharmaceutical Sciences, Idaho State University, Pocatello, ID[5]
The gut mucosal immune response is compromised in old rodents and monkeys, as well as in elderly humans. This diminished response reflects age-related deficits in (a) the lymphoid elements, e.g. local immunoglobulin production by plasma cells in the lamina propria, (b) the mucosal epithelial cells, e.g. reduced uptake and secretion of polymeric immunoglobulins by the polymeric immunoglobulin receptor (pIgR) or (c) both compartments. We initiated a qualitative and quantitative analysis of the sequence of events which culminates in the expression and function of the pIgR in rat hepatocytes in order to determine at which subcellular site(s) aging perturbs this process. The small decline in the steady state level of pIgR mRNA (<20%) suggests that aging does not impair transcription. An age-dependent lag in the incorporation of ^{35}S-cysteine into newly synthesized pIgR lends credence to the possibility that aging may affect this receptor at the level of translation. In vivo and in vitro data demonstrating age-related declines in the hepatic content and secretion of pIgA further implicate diminished translation in old animals. A concomitant loss of polymerized tubulin, indicative of fewer and/or shorter microtubules, suggests that the cytoskeletal-dependent translocation of the pIgR through posttranslational modifications (glycosylation, phosphorylation) and insertion into the plasma membrane may be impaired. The marked loss of pIgR from hepatocytes and reduced binding of polymeric immunoglobulin A (pIgA), both in vivo and in vitro, support this contention. The subsequent steps involved in the endocytosis and vesicular translocation of pIgR-pIgA complexes to the biliary pole appear to be impaired by aging based on quantitative autoradiographic (in vivo) and secretory (in vivo and in vitro) data. In summary, the age-related decline in epithelial cell uptake and transport of pIgA antibodies to the mucosal surface reflects, in part, perturbations in pIgR translation and translocation.

O 319 EXTENSION OF THE YEAST LIFE SPAN BY INDUCED EXPRESSION OF THE RAS2 GENE, Jiayan Sun, Shubha Kale, S.Michal Jazwinski, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112
The yeast *Saccharomyces cerevisiae* has a limited replicative life span, determined by the number of times an individual cell divides. It has been shown that certain genes are differentially expressed in an age-dependent manner, and defined alterations in the expression of specific genes can lead to a change in the life span of this organism. Expression of the v-Ha-*ras* oncogene under the control of a galactose-inducible promoter resulted in a 70% increase in the mean and maximum life span.

S. cerevisiae posesses two *ras* homologs, *RAS*1 and *RAS*2. We sought to determine if the *RAS*2 gene, a protooncogene, plays a role in yeast aging. To examine whether the expression of the *RAS*2 gene changes during the yeast life span, *RAS*2 mRNA levels from cells of different ages were subjected to Northern blot analysis. It appears that the expression of *RAS*2 gene decreases with age. Overexpression of this gene under the control of the *GAL*10 promoter led to an approximate 30% increase in the mean and maximum life span.

To determine whether the cyclic AMP pathway is involved in the life span extension by *RAS*2, cAMP was administrated to cells. Life span determination showed no difference with cAMP treatment. Caffeine, an inhibitor of phosphodiesterase, also failed to extend the yeast life span. Overexpression of *CYR*1 gene, coding for adenylate cyclase, under the control of a constitutive promoter (*ADH*) could not extend yeast longevity either.

Our results suggest that *RAS*2 gene plays an important role in yeast aging and its longevity-related function may not be mediated by cAMP pathway.

O 320 EFFECTS OF ELEVATED INTRACELLULAR CALCIUM ON GENE EXPRESSION IN CATECHOLAMINERGIC CELLS. Vyas S., Michel P.P., Anglade P., Ruberg M. and Agid Y., INSERM U289, Hopital de la Salpetriere, 75013 Paris, France.

The mechanisms of neuronal death in pathological aging of brain are not completely known as is the case for Parkinson's disease where there is a progressive and selective loss of dopaminergic neurons within the mesecephalon. In almost all types of cell death, disruption of calcium homeostasis leading to irreversible cell damage, is believed to be the final step of degenerative processes. The effects of increased intracellular Ca^{2+} produced by the ionophore A23187 in PC12 cells, differentiated by NGF, were examined. The treatment of cells with three to 10uM of ionophore A23187 for 24 hours resulted in neurite retraction and in the impairement of specific (3H)-dopamine uptake, however there was no significant loss of cell bodies. At lower concentrations tested, toxic effects were characterized by a reduction in dopamine uptake only. These effects were completely reversible upon the withdrawal of the ionophore. Similar treatment, but at higher concentrations (>30uM), caused widespread degeneration, and recovery process could not be observed. Electron microscopical studies revealed morphological changes in the cytoplasm, including formation of lipid droplets and reticulum vacuolation but no obvious nuclear alterations were detected. We also examined expression of tyrosine hydroxylase (TH) and of immediate early genes such as c-fos. The TH mRNA expression was significantly enhanced at low concentrations of the ionophore, at all time points examined (30 min to 24 hours). At concentrations >30uM and for a short incubation time (30 min) where no morphological changes were detectable, there was a decrease in TH mRNA levels reflecting changes in TH regulation. Longer treatments at these concentrations produced similar effects, most likely, in this case as a consequence of cell degeneration. Increased expression of c-fos mRNA was also observed shortly after initating treatment with low concentrations of the ionophore only. These results suggest that the modulation by calcium is biphasic and that there is a "set point" beyond which calcium exerts negative effects.

O 322 *Abstract Withdrawn*

O 321 TWO NOVEL SEQUENCE-SPECIFIC RNA BINDING PROTEINS ASSOCIATED WITH AGING HUMAN FIBROBLASTS

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Alteration in the control of general or regulated RNA processing events could potentially account for the multitude of changes observed in cellular aging. These alterations may be reflected in the level or activity of specific RNA binding proteins. In order to address this hypothesis, nuclear extracts were prepared from different passages of a human diploid fibroblast cell line, HS74. RNA-protein interactions were assayed by incubating a well characterized polyadenylation (PA) substrate RNA derived from SV40 (SVL) with these extracts. This RNA specifically associated with 3 proteins in Hela cell extracts (CstF-64, DSEF-1, and hnRNP C) and bound one protein non-specifically (hnRNP A) in UV crosslinking assays (Qian and Wilusz, MCB 11: 5312). Extracts prepared from early passage, serum starved or immortal HS74 cells showed a similar profile of RNA binding proteins. Late passage HS74 extracts, on the other hand, showed a dramatically different pattern of crosslinked RNA binding proteins. CstF-64, DSEF-1, and the hnRNP proteins were absent or greatly reduced. Two novel proteins of 38 and 45 Kd were the major crosslinked products. The RNA binding properties of the 38 and 45 Kd proteins were demonstrated to be sequence-specific using a series of deletion derivatives. We have currently mapped the binding site of the 38 Kd protein to a 25 base segment in the downstream element region of the PA signal. This segment contains a G-rich block followed by a 6 base U tract. Mutational analysis indicates that the G-rich block is also required for the binding of the 45 Kd protein. In summary, we have identified two novel sequence-specific RNA binding proteins which interact with a downstream element which influences processing efficiency of a model PA substrate RNA. We are currently assessing the significance of these proteins in cellular aging, as well as their importance to post-transcriptional regulatory mechanisms.

DNA, Telomeres, and Mitochondria

O 400 EVIDENCE FOR A CRITICAL TELOMERE LENGTH IN SENESCENT FIBROBLASTS, Richard C. Allsopp and Calvin B. Harley, Department of Biochemistry, McMaster University, Hamilton On., L8N 3Z5

Telomeres, the terminal G-rich sequences of eukaryotic chromosomes, shorten during aging of human fibroblasts *in vitro* and *in vivo*. We have previously examined the dependence of remaining replicative capacity on telomere length in 31 fibroblast strains established from various aged donors (Allsopp, R. *et al.*, Proc. Natl. Acad. Sci. **89** 10114 (1992)). Cultures were established from biopsies or surgical skin waste and DNA prepared from cells in the second passage (19 population doublings post-biopsy). HinfI/RsaI digested DNA was separated on agarose gels and telomeric DNA detected by hybridization to ^{32}P-$(C_3TA_2)_3$. Initial mean terminal restriction fragment length was found to be directly proportional to their *in vitro* replicative capacity (0.01 PDL/bp). Moreover, initial mean terminal restriction fragment length was a better predictor of replicative capacity than donor age. To further test the relationship between telomere length and senescence, we examined fibroblast strains established from progeria donors and aged-matched controls. Mean terminal restriction fragment length was significantly shorter in the progeria group (5.4 ± 0.5 kbp vs 8.8 ± 0.7 kbp), consistent with their reduced replicative capacity. We now show that the mean terminal restriction fragment length in senescent fibroblasts to be independent of *in vitro* lifespan and donor age (mean = 7.7 ± 0.4 kbp; age range = 0 to 90 years). Furthermore, inter-clonal variability in the amount of telomeric DNA was less at senescence than at early passage ($P < 0.1$). Together, these observations substantiate the effect of age on telomere length in humans, suggest telomere length is a biomarker of cell aging and support the hypothesis that the shortening of telomeres below a critical length can serve as a signal for cell senescence (Harley, C.B. Mut. Res. **256**, 271 (1991)).

O 401 INVESTIGATIONS OF THE ROLE OF MUTATIONS IN mtDNA AND MITOCHONDRIAL OXIDANTS IN AGING, Kenneth B. Beckman and Bruce N. Ames, Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720

The extranuclear somatic mutation theory of aging proposes that the accumulation of mutations in the mitochondrial genome, and the corresponding alteration of mitochondrial function, may be responsible in part for the phenomenology of aging. Recent studies of mtDNA deletions have suggested that they increase with age in humans and other mammals. Because of technical obstacles, there have been few studies of the accumulation mtDNA point mutations with age.

A method is being developed for the detection of mtDNA point mutations, called the Translational Arrest Assay (TAAssay). A fragment of restriction-endonuclease digested rat mtDNA is cloned into a TAA vector such that a functional, expressed fusion gene of the mtDNA and the α-fragment of β-galactosidase results. Frameshift and nonsense mutations in the continuous reading frame of the mtDNA result in lac^- bacterial colonies. The ratio of lac^-:lac^+ colonies is a measure of the frequency of somatic mutations, and an increase in the ratio with increasing age of the individual represents the accumulation of mutations over time. The technical details, results and implications of the use of the TAAssay are discussed.

The free radical theory of aging argues that oxidative damage (to DNA, proteins, and lipids) plays a key role in degenerative senescence. Amongst the numerous mechanisms known to generate oxidants, leakage of superoxide anion from the mitochondrial electron transport chain is of particular interest, since a correlation between the rate-of-living and life span has been observed.

Experiments have been initiated using yeast and human cells lacking mitochondria (ρ^0 cells) and their $mtDNA^+$ parent cell lines in an attempt to measure the role of *mitochondrial* oxidants in normal and pathological oxidative insults.

O 402 DEFECTIVE DNA REPAIR AS A POSSIBLE CAUSE OF AGING: STUDIES IN CONGENIC MICE, Michael E.T.I. Boerrigter, Yi Yin, Jan Vijg and Jeanne Y. Wei, Division on Aging at Harvard Medical School, and Gerontology Division, Beth Israel Hospital, Boston, MA 02115

Congenic mouse strains derived from mice with different lifespans are potentially useful in the investigation of the influence of genetic factors, such as DNA repair, on aging and age-related diseases. Chromosome 4 (CHR4) congenic mice are genetically identical to the long-lived C57bl/6 parental strain, with the exception of a small part of CHR4 which is derived from the shorter-lived Balb/c mouse strain. The recent localization of a DNA repair gene on the murine CHR4, within a region previously shown to affect lifespan, suggests that DNA repair may be an important factor influencing longevity, especially since Balb/c mice are relatively deficient in DNA repair.

Using a sensitive ^{32}P-postlabeling assay we measured the removal of benzo[a]pyrene (B[a]P)-induced DNA adducts, in liver DNA from the two parental strains, C57bl/6 and Balb/c, and 3 different CHR4 congenic strains. The rate of removal of the major B[a]P-adduct detected was more rapid in the parental C57Bl/6 strain and one CHR4 congenic mouse strain, B6.C-H-15^c, as compared with the parental BALB/c strain and the other two CHR4 congenic mouse strains, B6.C-H-26^c and B6.C-H-16^c. In senescent mice, the rate, but not the total amount, of DNA repair was decreased by about 50% in both parental strains.

The differential removal of DNA damage is likely indicative of an allelic difference in a DNA repair gene, present within the Balb/c region of both the B6.C-H-26^c and B6.C-H-16^c congenic mouse strains. Based upon healthful lifespan data, it might be determined whether aging and/or healthspan are influenced by allelic differences in this putative DNA repair gene.

O 403 DNA Damage and Repair in Telomeres

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Telomeric regions of chromosomes play an essential role in the maintenance of chromosomal stability. Telomeres may be 10 kb or more and consist of characteristic hexanucleotide repeating sequences. Decreases in telomeric length are seen with increasing donor age, and increasing passage in culture so that progressive telomeric shortening appears to be a significant biological marker of aging. Further, a unique RNA-containing enzyme, telomerase, is responsible for telomeric synthesis. Its activity appears to be restricted to germ cells, and is lost in somatic cells resulting in the progressive telomeric shortening with each round of replication. Yet, telomerase activity is re-expressed in tumor cells.

We have established a technique to study DNA damage formation and its repair in telomeres in human cell lines. Tissue culture cells are irradiated with UV, and the DNA is isolated and digested with restriction enzyme HinfI, and treated with T4 endonuclease V which cleaves DNA at pyrimidine dimer sites. The samples are separated by alkaline gel electrophoresis, transferred, and hybridized with endlabeled $(TTAGGG)_4$. Autoradiograms are scanned, and the data are transferred to a spreadsheet and analyzed for the frequency of pyrimidine dimers, and the repair. This technique and the data so far will be presented. Preliminary experiments indicate that there is little repair in the fibroblast telomeres.

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O 404 RAD9 HOMOLOGOUS TRANSCRIPTS ARE EXPRESSED IN HUMAN AND HAMSTER EXPONENTIAL FIBROBLASTS. Barbara A. Burkhart[1], Robert A. Schiestl[2], and J. Carl Barrett[1], [1]Lab of Mol. Carc., NIEHS, Research Triangle Park, NC 27709, [2]Dept. Mol. Cell. Tox., Harvard U., Boston, MA 02115

RAD9 of Saccharomyces cerevisiae controls the progression of the cell cycle following DNA damage. Loss of the gene results in failure to arrest the cell cycle before mitosis when DNA damage is unrepaired. This may result in genetic instability or increased mutation rate. We are interested in the role of RAD9-like genes in human and hamster and their potential involvement in cellular senescence and oncogenic progression.

A S. cerevisiae RAD9 gene (Schiestl, 1989) was used to probe exponential MRC5 human fibroblasts and Syrian hamster embryo cell total cDNA at reduced stringency. Two transcripts were detected in human exponential RNA which crosshybridized with the yeast probe. The larger transcript of 4.2 kb is approximately the same size as the yeast RAD9 transcript, a second crosshybridizing transcript was 3.7 kb. Exponential SHE cells had a single crosshybridizing transcript of 3.9 kb. RAD9-like expression was also examined in several transformed cell lines and a reduced transcript level was found in several lines of both human and hamster origin. Crosshybridizing sequence has been detected in both human and SHE cDNA libraries and we are currently screening for clones.

O 405 DNA POLYMERASE α ACCESSORY PROTEIN (αAP) ENHANCES DNA BINDING AND ACTIVITY OF ENZYME ISOLATED FROM AGED HUMAN DONORS, David Busbee, Matthew Schroeder, Vinod Srivastava and Susan Miller, Department of Anatomy and Public Health, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

DNA polymerase α (pol α) isolated from fibroblast lines established from aged human donors (AG3529) exhibits decreased DNA binding and decreased activity compared with fetal human fibroblasts (WI38), and neonatal human fibroblasts (GM8333). WI38, GM8333 and AG3529 cells transformed with pSV3.neo, an SV40-derived plasmid expressing large T antigen (TAg), exhibit pol α with covalently bound TAg which has high specific activity and exhibits high affinity of binding to synthetic oligonucleotide templates and to native DNA templates. Transformed WI38 (2RA) shows two pol α peaks, both of which have approximately the same specific activity as the parent cell line. Transformed GM8333 (M7222) exhibits pol α which is TAg positive and is slightly higher in specific activity than pol α from GM8333. Transformed AG3529 (2-1) shows a single pol α peak which has about 15-fold higher specific activity and significantly higher DNA binding affinity than the parent cell line pol α. Pol α from these cell lines was treated with DNA polymerase α accessory protein (αAP), isolated from the mouse transformed cell line L1210. Enzyme from WI38, 2RA, L1210, and 2-1 showed no enhanced DNA binding and increased specific activity in the presence of αAP. However, pol α from AG3529, established from a 66 year old donor, showed significantly increased binding affinity for DNA in the presence of αAP, and exhibited about a 6-fold increase in enzyme activity. We propose that a decrease in binding of αAP to pol α in old cells may account for a loss of pol α DNA binding affinity and specific activity which, in turn, may be associated with the G_1/S replication block seen in aging cells. Preliminary data suggest that this loss of αAP-pol α binding may not be associated with decreased expression of pol α, but with decreased expression of αAP. Supported in part by AG06347 and by a research enhancement award from Texas A&M University.

O 406 TELOMERE LENGTH AS A BIOMARKER FOR CELL TURNOVER IN NORMAL AND ATHEROSCLEROTIC TISSUE FROM HUMANS, Edwin Chang and Calvin B. Harley, Department of Biochemistry, McMaster University, Hamilton, Ontario, CANADA, L8N 3Z5

Repeated denudation of the endothelium and consequent senescence of intimal cells may contribute to atherogenesis. Our previous work has shown that telomeres, the ends of eukaryotic chromosomes, shorten with *in vitro* and *in vivo* age of human skin fibroblasts (Harley et al., Nature **345**: 458 (1990), Allsopp et al. Proc.Natl.Acad.Sci. **89**:10114 (1992)). Our aim was to determine if telomere length serves as a biomarker of cellular turnover in tissues implicated in atherogenesis. Telomere lengths were assessed by southern analysis of terminal restriction fragments (TRF) in *HinfI*/*RsaI*-digested genomic DNA. Mean TRF length decreased with *in vitro* age in endothelial cell cultures from human umbilical veins (slope (m) = -190 base pairs (bp)/Population Doubling (PD), P=0.01), iliac arteries (m = -120 bp/PD, P=0.05) and iliac veins (m = -180 bp/PD, P=0.05). When mean TRF length was assessed for endothelial cells cultures from donors of different ages but of the same passage, there was a statistically significant decrease for iliac arteries (m=-102 bp/yr, P=-0.01) but not for iliac vein (m=-47 bp/yr, P=-0.14). This is consistent with increased cellular turnover *in vivo* of endothelial cells from iliac artery. Preliminary data also show a significant decrease in mean TRF length of intimal (P=0.03) and medial (P=0.05) tissue as a function of donor age thus demonstrating cell turnover and/or expansion *in vivo* of primary vascular tissue. Intimal tissues from one individual who displayed extensive development of atherosclerotic plaques had mean TRF lengths close to those observed for senescent endothelial cells *in vitro* (~6 kbp). In general, mean TRF lengths of medial tissues from plaque regions were shorter than those from non-plaque regions. Thus telomere length may be a biomarker for the replicative history and capacity of intimal and medial tissue. Our data also support a role for focal replicative senescence of vascular tissue in atherogenesis.

O 407 ARE SOMATIC CELLS INHERENTLY DEFICIENT IN METHYLATION METABOLISM? A PROPOSED MECHANISM FOR DNA METHYLATION LOSS, SENESCENCE AND AGING. Craig A. Cooney, Biology Department, Beckman Research Institute, City of Hope, Duarte, CA 91010 USA

A mechanism of aging is proposed for mammals and other vertebrates. In this mechanism, somatic cells have inherent deficiencies in methylation metabolism with respect to their capacity to methylate DNA. This leads to incomplete DNA methylation in each cell cycle which, accumulated over many cell cycles, contributes to genetic instability, senescence and cancer. These proposed metabolic deficiencies are present from the time somatic cells are young. The deleterious effects of these deficiencies are mainly realized after many cell divisions and after most individual animals in nature have reproduced and been subject to predation. These deficiencies evolved in animals eating a balance of nutrients from nature.

Evidence from the literature is reviewed which establishes that methylation is lost from the DNA of mammalian somatic cells as they age both *in vivo* and *in vitro*, and that DNA methylation levels are influenced by factors, such as diet, which affect methylation metabolism. Partially correcting the proposed deficiencies is considered as a possible molecular mechanism by which caloric restriction extends life span. Other possible dietary and transgenic means to correct the proposed deficiencies and extend lifespan are discussed.

O 408 IMMUNOLOGICAL STATUS AND GENOMIC STABILITY/INSTABILITY IN CENTENARIANS

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Centenarians were considered a rare curiosity. This is no longer true. First, their number is dramatically increasing. Indeed, in Italy there were 49 at the beginning of this century, and nowadays there are more than two thousand (1 out of 5-10,000 people). It can be predicted that the number of people aged >95 or 100 years will represent in the near future a consistent part of the old population. Second, centenarians are the best example of people who escaped the above mentioned major age-related diseases. It can be predicted that, in order to reach the extreme limit of human life, centenarians should be equipped with a combination of very well preserved defense mechanisms, and that they are the result of optimal combination of an appropriate lifestyle plus genetic background. However, the literature about the basis of human longevity is surprisingly scanty, and no systematic studies exist on biochemical, hematological, nutritional, immunological and neuroendocrino-logical parameters of people of far advanced age. In last years, we have studied the functionality of their immune system, and we have found i) a significant decrease of peripheral blood lymphocytes (PBL); ii) no modifications of the percentage of the main T cell subsets (CD3+, CD4+ or CD8+ cells), but a decrease of their absolute number/mmc; iii) a well preserved ability of T cells to proliferate after mitogen-stimulation; iv) a dramatic decrease of peripheral B lymphocyte (CD19+), accompanied by an increase of IgG, IgA, IgG1, IgG2 and IgG3 serum level and an unexpected very low incidence of organ-specific autoantibodies; v) a significant increase of cells with NK markers, and a well preserved NK activity; vi) a significant increase of an HLA aplotype; vii) an extremely well preserved PBL capability to cope with oxidative stress.

The study of spontaneous and bleomycin-induced chromatid breaks, spontaneaous and mitomycin-C micronuclei formation, sister chromatid exchange suggests that a complex situation is present in centenarians as far as genomic instability is concerned.

O 409 AGE-ASSOCIATED ACCUMULATION OF RAT MITOCHONDRIAL DNA DELETIONS.

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Large sized (several kb) mitochondrial DNA (mtDNA) deletions have been reported to occur, albeit infrequently, both with ischemia and aging in humans. A significant accumulation of mtDNA alterations may promote cellular degeneration and death by interfering with target gene function.

In order to investigate whether aging is associated with increased levels of small mtDNA deletions, we performed primer shift PCR on DNA extracted from brain tissue of weanling (4 week), young adult (4-5 month) and old (24 month) Fisher 344 rats. Primers were selected based upon published rat mtDNA sequences to permit selective stepwise amplification of nine 1 kb intervals from the 7,000 to 16,000 bp regions of the mtDNA genome. We found reproducible age-related amplification deficits in two of the nine 1 kb regions. These were located in the 9-10 kb and 11-12 kb intervals, consistent with 100-200 bp deletions.

Thus, in the rat model of aging, there are multiple, nonrandom, smaller mtDNA alterations that occur commonly and with increasing frequency during aging.

O 410 MAGNESIUM INHIBITS NON-ENZYMATIC GLYCOSYLATION OF DNA: IMPLICATION ON AGING PROCESS.

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All reducing sugars react non-enzymatically with amino groups of protein to form advanced glycosylation end (AGE) products. AGEs have been implicated in the pathogeneses of aging including diabetes, cardiovascular and renal diseases. Glucose, one of the reducing sugars, has also been shown to react non-enzymatically with DNA to produce significant structural and biological alterations. Fructose, another naturally occurring abundant reducing sugar, although has only been tested to react with proteins, could also be a potent reactant to DNA. Interestingly, a recent study reported that the fructose-fed human subjects had higher fecal excretion of magnesium than the glucose-fed subjects (Ivaturi R and Kies C. Plant-Foods-Hum-Nutr 1992; 42: 143). Magnesium is also intimately involved in many metabolic processes including nucleic acid synthesis and stability. In an attempt to ascertain chemical interactions between fructose,magnesium and nucleic acids a preliminary study was therefore designed to follow the non-enzymatic glycosylations of DNA by glucose and fructose in presence and absence of magnesium. DNA (10mM) was incubated with glucose 6-phosphate (G-6-P) and with fructose 6-phosphate (F-6-P), at a concentration of 150 mM. The occurrence of structural changes in the DNA molecule, after four days of incubation, was evaluated by the UV spectroscopy and showed substantial changes in the UV absorbance in the 300 to 400-nm range due to glycosylation by F-6-P whereas slight changes occurred due to G-6-P. However, when the concentration of G-6-P was increased to 450 mM (300%) moderate spectral changes were noted. Addition of 150 mM $MgCl_2$ significantly reduced the spectral changes, most likely by inhibiting glycosylation of DNA. From these preliminary data it appears that the glycosylation of DNA by fructose is stronger than by glucose which can be prevented by the presence of magnesium. Additional studies are necessary to understand the mechanisms of their effect on aging.

O 411 Age and Dietary Restriction Mediated Alterations in the Levels of DNA Binding Proteins

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It has been proposed that the age-related decrease in gene expression plays an important role in the physiological decline observed in the aging organism by impairing the organism's ability to respond to environmental stimuli. Recent evidence suggests that transcriptional regulation plays a critical role in this age-related effect. To begin the study of this problem, we chose to examine five previously characterized transcription factors in hepatocytes isolated from young (4-6 month) or old (24 month) male Fischer 344 rats. In addition to using *ad libitum* fed animals, we also isolated and examined hepatocytes from rats fed a calorie restricted diet that increased both maximum lifespan and life expectancy. The use of hepatocytes yields a relatively homogeneous cell population and thus allowed us to generate extracts that were highly reproducible. In preliminary results obtained from a small numbers of animals, we generated the following data:

The DNA binding activity of the basal transcription factor TFIID (or TBP) and SP1, a ubiquitous transcription factor are not altered by either age or dietary restriction. The binding activity of AP-3, a factor involved in the transcription of SV40, is reduced by dietary restriction in both young and old animals, but there is no age effect. NF-κB site binding, which is normally induced by both free radicals and by tumor promotors such as TPA, is reduced both by age and by dietary restriction. In contrast, AP-1 binding, which is also stimulated by free radicals and tumor promotors is increased with age, but only in the *ad libitum* fed animal. Together, these results show that both aging and dietary restriction modulate the levels of some but not all transcription factors. These results on AP-1 and NF-κB suggest that there are at least two different regulatory mechanisms for sensing oxidation/free radical damage and responding to this damage by altering transcriptional regulation. It is interesting to hypothesize that the difference between the nuclear site of activation of AP-1 and the cytoplasmic site of activation of the NF-κB family of factors may be critical to the observed differences. We will present these preliminary studies as well as the results of studies now in progress on larger numbers of animals.

O 412 MITOCHONDRIAL ACTIVITIES DURING AGEING IN THE RAT.
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The mitochondrial theory of ageing is gaining support in view of the increasing mitDNA deletions accompanying senescence. MitDNA codes for a total of 12 polypeptide chains belonging to the membrane sectors of respiratory complexes 1, II, IV and of ATP syntethase. We have therefore investigated the corresponding, besides others, enzymatic activities of mitochondria from different tissues of rats between 2 and 26 months of age. No major changes of enzymatic activities and Coenzyme Q and cytochrome content were observed in liver and kidney mitochondria with ageing; in different mitochondrial fractions from selected areas of the brain periodical changes were observed, but no significant decline occurred in the old rats. On the other hand a dramatic fall of NADH cytochrome c reductase occurred in skeletal and cardiac muscle. This activity represents the integration of Complex 1 and Cmplex III activity through the Coenzyme Q pool; since Complex III activity and coenzyme Q are unchanged, the decrease of NADH cytochrome c reductase has to be attributed to loss of Complex I activity. Unfortunately, NADH cytochrome c reductase could not be assayed in other tissues because of the strong activation of rotenone-insensitive NADH oxidation, therefore we do not know whether similar declines are observed in other tissues. Complex I contains 6 out of the 12 polypeptide chains encoded by the mitochondrial genome, and this may explain its preferred decline compared with other activities. Since complex I activity may be rate-limiting in the overall respiratory chain, its fall would contribute to a major energy defect in the mitochondria of senescent cells. Contrary to other investigators who found a decrease of Coenzyme Q content in tissues from aged animals, we have been unable to notice significant decrease in the isolated mitochondria from any of the tissues investigated.

O 413 MITOCHONDRIAL DELETIONS WITH AGE IN THE NEMATODE *C.ELEGANS.*

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C.elegans is an excellent model for examining the aging process due to its short life span, ease of handling and well characterized genetics. Further, the availability of mutants which increase the life span extend the amenability of this animal to fundamental studies regarding aging.

In various mitochondrial myopathies, high levels of mitochondrial deletions are assumed to be causal to these conditions. Recently, deletions in the mitochondrial genome have been identified which increase with age. Mosaicism of deletions in various tissues has also been demonstrated with increasing age. We have examined *C.elegans* for mitochondrial deletions with increasing age to determine if this phenomena was widespread througout metazoans. In addition, it is hoped that through examination of mitochondrial deletions in individual cells in *C.elegans*, functional consequences can be inferred.

PCR primers specific for a 4.3kb region of the mitochondrial genome were used in order to identify potential deletions with age in a number of strains of *C.elegans*. This approach identified several deletions which increased as a function of age. By sequencing these deletions a number of direct repeats were identified at the breakpoints. The abundance of these deletions was then determined by quantitative PCR with regard to the mitochondrial genome.

O 414 CLONING OF NUCLEAR GENES AFFECTING THE LIFE SPAN OF THE FILAMENTOUS FUNGUS PODOSPORA
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In the filamentous fungus *Podospora anserina* the onset of senescence is controlled by both nuclear as well as extranuclear genetic traits. Among the genetic factors analyzed so far extranuclear factors (mitochondrial DNA, mitochondrial plasmids) have been characterized in detail on the genetical and the molecular level. However, since not a single nuclear gene affecting the life span of *P.anserina* has been cloned so far, only very little is known about the molecular characteristics of these genetic factors.
Here a strategy towards the cloning of the nuclear gene *grisea* is described. Mutation of this gene has a pleiotropic effect on *P.anserina* cultures resulting in changes of the morphology (e.g. reduced production of aerial hyphae, reduced fertility) as well as the life span (39 days vs 24 d of the wild strain). Using a combination of formal genetic approaches (linkage analysis) and molecular techniques (e.g. pulsed field electrophoresis, heterologous transformation) gene *grisea* was assigned to the smallest of seven physically fractionated chromosomes (chromosome I) of *P.anserina*. Chromosome I was utilized to construct a chromosome specific genomic cosmid library which subsequently was used to transform mutant *grisea*. A number of wild-type transformants were selected which are currently analyzed in detail. From one of these transformants a single cosmid clone was isolated containing an insert of about 40 kbp which is derived from the wild-type *P.anserina* genome. In order to identify and characterize a specific gene which gives rise to the complemention of mutant *grisea*, the inserted sequence will be shortened to a minimal length and the corresponding plasmids will be analyzed for their biological activity (complementation of mutant *grisea*). Finally, the identified sequence can be utilized as a specific gene probe to clone and characterize the corresponding mutant gene of long-lifed mutant *grisea*.
Data derived from the described analysis can be expected to provide novel clues about the role of specific genes (longevity genes) affecting the life span not only in this particular model system but most likely may also be of relevance for higher biological systems.

The experimental work was supported by a grant of the *Deutsche Forschungsgemeinschaft* (Bonn-Bad Godesberg) to the author.

O 415 LEVELS OF GENE SPECIFIC REPAIR DURING G_1 PHASE
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Repair of DNA damage, induced by the chemotherapeutic agent cisplatin, has been measured by a new highly sensitive assay. Single stranded DNA, capable of hybridizing to gene specific probes, is enzymatically generated by T4 DNA polymerase. In the presence of cisplatin or H_2O_2 induced lesions, the 3'-5' exonuclease activity of T4 DNA polymerase is inhibited and the formation of single stranded DNA is prevented; thus lowering the level of probe hybridization. A significant difference in the hybridization profiles for human DNA probed for specific genes, from synchronized G_1 phase cells, treated with either 50 or 100 mM cisplatin, has been measured. The rate of repair in the non active δ-globin gene has been compared to that in the active DHFR gene. During early G_1 phase, the DHFR gene was efficiently repaired within 2 hours, while there was no measurable repair in the δ-globin gene with 5 hours.

We are measuring gene specific repair of H_2O_2 induced DNA damage in early passage, late passage, and SV40 transformed human diploid fibroblasts during G_1 phase. Using DHFR and δ-globin gene probes, the effect of cell age on repair rates, following oxidative damage, is being investigated.

O 416 THE DETECTION OF DNA NEUROADDUCTION IN AGING, J.J. Steinberg, Gary W. Oliver, Jr., Antonio Cajigas, Peter Davies, Departments of Pathology, Neuropathology, and Neurosciences, Albert Einstein College of Medicine, Bronx, NY 10461

We have reported (UCLA Symp. vol. 123: 53-65, 1990) that increased DNA damage and its incomplete or inadequate repair may be important in the degenerative changes evident in neuronal aging, Senile Dementia of the Alzheimer's type (SDAT) and Down's syndrome. The consequence, therefore, of altered DNA repair in SDAT may be mechanistically involved in accelerated neuronal death or decline. To document the pattern of DNA neuroadduction in aging, we extracted DNA from frontal, parietal, and caudate areas of five brains rapidly harvested at demise from elderly patients (mean = 74 y/o) with SDAT. The assay for neuroadduct detection and normal base composition is carried out by radiolabelling DNA with all four deoxynucleotides (dNMP) through nick translation which demonstrates that SDAT labels five fold greater than control DNA (human placenta, human liver, calf thymus, and HeLa DNAs). A subsequent 3'-monophosphate digest radiolabels representative dNMP's and neuroadducts. Separation occurs in two dimensional PEI-cellulose thin-layer chromatography (2D-TLC) - which resolves dNMP's. Scintillation detection, densitometry and phosphoroimaging correlate and accurately reflect dNMP content. Results demonstrate that neuroadduction in frontal and parietal areas correlate well, and caudate less so. Persistent amounts of uracil (as the deoxynucleotide - dUMP) appear in each - and though a possible product of dCMP deamination - are not apparent in control DNAs. An additional triad of neuroadducts appear approaching the front, with X,Y axes coordinates at 3,12 cm and beyond - but are not consistently present in all brains or all areas. Also, an additional neuroadduct specie(s) is present beyond dCMP, and may represent methyl- or hydroxymethyl-dCMP. An occasional small adduct about the R_f for 8-hydroxy-dGMP is also demonstrable - but not consistent. An excess of dNMP fragments are constant in SDAT - and not persistently evident in other human, frozen or degraded tissue. These findings further support the possibility of diminished DNA repair or increased injury in aging and/or SDAT. These techniques quantify low molecular weight neuroadducts in DNA, and may serve as a biomarker or dosimeter of aging, or SDAT effect.

O 417 LOSS OF TELOMERIC DNA DURING AGING OF NORMAL AND TRISOMIC HUMAN LYMPHOCTYES. Homayoun Vaziri and Calvin B.Harley, Department of Biochemistry, McMaster University, Hamilton, On., L8N 3Z5

The telomere hypothesis of cellular aging proposes that loss of telomeric DNA (TTAGGG) from human chromosomes may ultimately cause cell cycle exit during replicative senescence. Since lymphocytes have a limited replicative capacity, we wished to determine whether accelerated telomere loss is associated with the premature immunosenescence of lymphocytes in individuals with Down Syndrome (DS), and whether telomeric DNA is also lost during aging of lymphocytes *in vitro*. To investigate the effects of aging and trisomy 21 on telomere loss *in vivo*, genomic DNA was isolated from peripheral blood lymphocytes of 140 individuals (0-107 y) and 21 DS patients (0-45 y). Digestion with restriction enzymes *Hinf*I and *Rsa*I generate terminal restriction fragments (TRFs) which can be detected by southern analysis using a telomere-specific probe, (^{32}P-$(C_3TA_2)_3$). The rate of telomere loss was calculated from the decrease in mean TRF length as a function of donor age. DS patients showed a significantly higher rate of telomere loss with donor age (133±15 bp/y) compared to age-matched controls (41±7.7 bp/y) ($P<0.0005$), suggesting that accelerated telomere loss is a biomarker of premature immunosenescence of DS patients and may play a role in this process. Telomere loss during aging *in vitro* was calculated for lymphocytes from two normal individuals grown in culture for 20-30 population doublings. The rate of telomere loss was ≈90 bp/cell doubling comparable to that seen in other somatic cells. Telomere lengths of lymphocytes from centenarians and from older DS patients were similar to that of senescent lymphocytes in culture, which suggests that replicative senescence could partially account for aging of the immune system in DS patients and elderly individuals.

O 418 **AGE-DEPENDENT CHANGES IN TELOMERIC HETEROCHROMATIN,**

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All human chromosomes terminate with a 5 to 15 bp track of $(TTAGGG)_n$ telomeric DNA. Telomere lengths in many somatic cells are reported to progressively shorten *in vivo* and *in vitro* with senescence. It has been hypothesized that this decline in telomere length is causally related to senescence. If true, then telomeres may alter cell life span via changes in telomeric chromatin structure. We have tested this proposed mechanism by probing nuclei with novonase, a chicken endonuclease that preferentially digest active gene regions. When blots containing DNA from novonase digested HeLa nuclei were probed with the telomere repeat, telomere sequences were found to be preferentially resistant to novonase in comparison to bulk DNA, suggesting that novonase is a good probe of telomeric heterochromatin. We then compared the accessibility of the telomeres to novonase as a function of replicative senescence in MRC-5 fibroblasts. When blots containing DNA from novonase digested nuclei of early- versus late-passage fibroblasts were probed with the TTAGGG repeat, the telomere sequences in late-passage cells were clearly more sensitive to novonase digestion than were the telomeres in early-passage MRC-5 or immortalized HeLa cells. These results suggest that chromatin structure of the telomere region is becoming more accessible to protein binding as telomeres shorten in late-passage MRC-5 cells. We propose that the changes in telomeric chromatin structure that accompany telomere shortening alter the expression of genes in the subtelomere region and thereby induce cellular senescence.

O 419 HETEROGENEOUS METHYLATION OF LIVER DNA IN AGING RATS, ZONG-Yu ZHANG, ZB MAO, XS MAN and TJ TONG, Department of Biochemistry, Beijing Medical University, Beijing, 100083, PR China

DNA isolated from rat liver of various ages was digested with restriction endonuclease HpaII and MspI. The digested DNA was electrophoresed in agarose gel. It was showed that DNA fragments digested with MspI consisted of a strong band with the size of 4.8 Kb and a weak band of 0.1 Kb, while DNA fragments with HpaII consisted of a strong band with the size of 20Kb. It was estimated that approximatly 80 % of the CCGG sequence was methylated with no changes related to ages. No difference of DNA methylation of rat liver between aging and young rats was shown by reverse-phase high performance liquid chromatography after DNA treated with nuclease P1 and alkaline phosphatase also.

Further study using micrococcal nuclease to seperate active and inactive chromatin of liver, then isolated DNA from them, showed that transcriptionally active DNA from liver of aging and young rats contained less 5mC than inactive DNA from same rats. However it is interesting to show the result that the aging one significantly reduced level of 5mC in transcriptionally active DNA, but elevated level of 5mC in inactive DNA. It suggests the difference of methylation may exist in different constitutional parts of DNA between young and aging one.

Late Abstracts

THE CALPAIN-CALPASTATIN SYSTEM AND BAND-3 PROTEIN DEGRADATION IN RED CELLS OF OLD AND YOUNG INDIVIDUALS, Tova Glaser, Neta Schwarz-Benmeir, Zvia Bar-Noy and Nechama S. Kosower, Department of Human Genetics, Sackler Institute of Medicine, Tel-Aviv University, Israel.

Little is known about red blood cells (RBC) in relation to aging of the whole organism. We found that degradation of band-3 protein, the major transmembrane glycoprotein, by calpain I (a catalytic μM Ca requiring thiol proptease) was enhanced in RBC membranes of old individuals (>70 years) as compared to young (20-30 years old). Degradation was enhanced by membrane alkylation (by mBBr or NEM) to the same extent. However, DIDS (the anion exchange inhibitor) enhanced calpain degradation in the young only. Increased degradation in the old as compared to young was also observed in membrane vesicles containing the band-3 95 kDa domain and its chymotrypsin generated fragment CH-60. The 43 kDa cytoplasmic domain was degradeted by calpain to a similar extent in the old and young. As shown by calpain antibodies, little enzyme was membrane associated in both RBC types. Increased cellular Ca, induced translocation of calpain to the membranes. Activation of calpain added to membranes as well as degradation of band-3, was greater in the old than in the young. In addition, the cytosol of RBCs from old contained less of the endogenic inhibitor, calpastatin, than cytosol of the young individuals. Membranes from old were demonstrated by antibodies to contain less calpastatin than young. Our results indicate enhanced calpain translocation and activation, resulting from irreversible loss of calpain from the old. That band-3 from the old is more efficiently degraded by calpain, suggests age related modifications in the protein and/or other membrane components plus alterations in the calpain-calpastatin system.

BCL-2 TRANSGENE INHIBITS NEUTROPHILS CELL DEATH BUT NOT THEIR ENGULFMENT BY MACROPHAGES, Eric Lagasse and Irving L. Weissman, Department of Pathology and Developmental Biology, Stanford University School of Medicine, Stanford, CA94305. Neutrophils, the commonest type of leukocytes, have a limited life span in circulation. They undergo programmed cell death, a process leading to their recognition by macrophages in the liver and spleen. In addition neutrophils have a major role in initiation and amplification of inflammation. Normally, acute inflammation resolves with minimal residual tissue damage, but the removal of neutrophils from inflamed site is a prerequisite for resolution to occur. Histological observations show that engulfment by macrophages is an important disposal route, and changes in the senescent neutrophil have been associated with their recognition by macrophages. To assess the role of programmed cell death of neutrophils in this recognition event, we generated transgenic mice using a myeloid-specific promoter that redirected bcl-2 expression from early myeloid progenitors in bone marrow to mature neutrophils in blood. Bcl-2 protected neutrophils from apoptosis. Despite this, myeloid cells homeostasis is essentially unaffected by bcl-2, since both the total number of myeloid cells in bone marrow, spleen and blood is the same in bcl-2 and nontransgenic siblings. Moreover, macrophages uptake of neutrophils expressing bcl-2 still occurs. This transgenic model indicates that the pathway which trigger phagocytosis of aging neutrophils can be distinguished from programmed cell death.

TRANSGENIC MICE OVEREXPRESSING SE-GLUTATHIONE PEROXIDASE IN THE BRAIN: DIFFERENTIAL RESISTANCE TO MPTP-MEDIATED NEUROTOXICITY, Marc-Edouard Mirault, Alain Tremblay, Geneviève Trépanier, Denis Furling and Jack Puymirat. Ontogénèse et génétique moléculaire, CHUL Research Center and Laval University, Québec, P.Q., Canada GIV 4G2.

There is accumulating evidence for a role of normal aging and free radical-mediated oxydative damage in the pathogenesis of several neurodegenerative conditions including Alzheimer's and Parkinson's diseases. Toxic radicals such as $^{\circ}OH$ eventually derived from $O_2^{\cdot-}$ and H_2O_2 generated by neurotoxins or endogenous processes may act by triggering peroxidation in membrane lipids, and inducing oxydative damage to proteins and DNA. Seleno-glutathione peroxidase (GSHPx) and GSH, an enzyme and cofactor that can reduce both H_2O_2 and fatty acid hydroperoxides, may be key components of the cellular antioxidant defense system (Mirault *et al., J. Biol Chem.* **266**, 20752, 1991). In order to evaluate the role of GSHPx as protective agent against oxidative injury in cells of the Central Nervous System, we have generated transgenic mice that overexpress GSHPx constitutively in the brain. We are currently comparing transgenic versus syngenic non-transgenic mice 1) for the patterns of GSHPx expression in various regions and cell types of the brain; 2) for the relative resistance of dopaminergic nigrostriatal cells to the *in vivo* neurotoxicity of MPTP; 3) for the relative resistance and growth properties of cholinergic and catecholaminergic neuronal cells cultured *in vitro*, following (or not) exposure to various agents and conditions that generate radicals, including MPP^+ and 6-OHDA. Results to be presented indicate that the GSHPx-overexpressing mice are notably more resistant to MPTP-mediated neurotoxicity than syngenic non-transgenic mice.

MICE SELECTED FOR INCREASED LENGTH OF REPRODUCTIVE LIFE, Parviz M. Sabour and Jiro Nagai, Centre for Food and Animal Research, Agriculture Canada, Ottawa, Ontario, Canada K1A 0C6

To study molecular aspects of aging, lines of mice for selected increased length of reproductive life and unselected controls were developed from foundation stocks of diverse origin (Nagai et al., Theor. Appl. Genet. 79:268-272, 1990). In two selected lines, one with litter size standardized to eight at birth and the other without standardization, litters of the 5-7th parity were used for line propagation. The unselected control line was maintained by breeding the first litters every generation so that the number of generations in the selected and control lines differed over the duration of the experiment. In the three lines, breeders were pair-mated at 7 weeks of age and were kept cohabited throughout their reproductive life. The number of breeders per line was designed to result in similar inbreeding in the selected and control lines. The contemporary selected lines at generation 12 and control line at generation 34 were compared for lifetime performance by keeping breeders as long as they produced progeny. Breeders of the selected lines had, on average, significantly more (ca. 50%) reproductive days; larger (ca. 60%) number of parturitions, and thus, a larger number of progeny during lifetime than those of the control lines. It was concluded that selection was effective in increasing the length of reproductive life and production of progeny during lifetime. Differences in DNA fingerprinting patterns between the selected and control lines were demonstrated.