MOLECULAR TOXICOLOGY

Organizers: Spencer Farr and Armen Tashjian

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Poster Sessions

Molecular Toxicology

Opening Address

A5-001 CURRENT TOXICOLOGY PRACTICES: LIMITATIONS AND OPPORTUNITIES FOR NEW METHODOLOGY, James T. MacGregor, Toxicology Laboratory, SRI International, Menlo Park, CA 94025

The basic methodology of safety evaluation has changed little during the past 40 years (Barnes, J.M. and Denz, I.A., *Pharmac. Rev.* **6**, 191-242, 1954). Systemic toxicity has been evaluated primarily by using four principle parameters as indicators of organ damage laboratory animals: 1) gross clinical observations, 2) organ and body weight changes, 3) histological alterations in tissues, and 4) hematological and clinical chemistry parameters. Evaluation of genotoxic potential has been hampered by the lack of assays suitable for determining DNA alterations in animal tissues *in vivo*, and has relied heavily on extrapolation from *in vitro* assays and specific endpoints that can be measured easily only in restricted tissues *in vivo*. Evaluation of carcinogenic potential by histological identification of tumors is so labor intensive that only about 500 of the many thousands of chemicals to which humans are exposed routinely have been tested in a cancer bioassay that would be considered adequate by current testing standards. However, in recent years major scientific progress in understanding the molecular events associated with cell death and neoplastic transformation has created an opportunity for improved methods of evaluating cellular toxicity, genetic damage, and neoplastic transformation. The realization that cells express specific gene-inducible responses to major classes of cellular damage such as alterations in cellular protein structure, introduction of adducts or strand-breaks into DNA, heavy metal exposure, oxidative damage, and intracellular free radical formation, suggests that simple assays for expression of these damage-specific gene families could serve as sensitive indicators of cellular toxicity. Additionally, alterations in base sequences and expression of oncogenes can now be used as markers of genotoxic damage and pre-neoplastic transformation *in vivo*. Specific opportunities for improved methods include: (1) the use of damage-specific gene responses to monitor cellular damage, 2) monitoring the genetic integrity of specific oncogenes as biomarkers of genetic damage and neoplastic transformation, 3) the use of transgenic animals with human metabolizing genes as improved models for *in vivo* studies, and 4) increased capability to measure gene mutations in organs and tissues *in vivo*. Integration of these methodologies into routine toxicology evaluations can be expected to lead in the near future to a greatly improved ability to predict human health effects from laboratory animal data.

Molecular Mechanisms of DNA Repair and Recombination

A5-002 DNA REPAIR PATHWAYS: RELATIONSHIPS WITH TRANSCRIPTION, Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

Multiple strategies have evolved to reduce the genotoxic consequences of endogenous and environmental agents that damage DNA. Unrepaired damage can interfere with the essential transactions of replication and transcription, leading to mutation and dysregulation of gene expression. The most ubiquitous and general repair mechanism, that responds to a variety of types of DNA lesions, is nucleotide excision repair. Recent studies have identified a subpathway in which an RNA polymerase blocked at a lesion, with the aid of additional factors, may recruit repair enzymes to achieve preferential repair of the transcribed DNA strand. This transcription-coupled repair (TCR) pathway is also ubiquitous and it has important implications for carcinogenic mechanisms. Our current understanding of the mechanism of TCR will be discussed (**1, 2**).

Several genes implicated in Cockayne syndrome (CS) are required for TCR but not for overall genomic repair. Although the victims of CS are sensitive to sunlight they are not cancer prone. In contrast, the victims of xeroderma pigmentosum (XP), are extremely susceptible to UV-induced skin cancer. In complementation group C (XPC), the TCR pathway is intact but repair of the overall genome is defective. Perhaps one or more genes at risk in the pathway to malignancy are not expressed in quiescent cells and therefore not accessible to repair in XPC. Damage may accumulate in such genes until the cells are activated to enter a proliferative state (e.g., by tumor promoters), by which time it may be too late for TCR to clear the genes of lesions before DNA replication results in mutation at the damaged sites.

Mutations in the p53 tumor suppressor gene have been found in a majority of human tumors. Analyses of the spectrum of p53 mutations show a bias for mutations originating from lesions presumed to have been in the non-transcribed strand of the gene in certain tumor types, consistent with strand specificity for the formation and/or repair of DNA damage. The reported preferential repair of the transcribed strand of the p53 gene may account for the resulting mutational spectra of this gene in skin tumors from presumed XPC patients (**3, 4, 5**).

Gratuitous TCR might occur where transcription is inhibited by DNA sequence-dependent secondary structures. These sites might be mistakenly treated as lesions by the TCR system. Reiterative DNA turnover could eventually give rise to mutations as a consequence of repair replication errors. Such nucleotide sequence changes in particular target genes could then contribute to carcinogenesis.

(**1**) Hanawalt PC, *et al.* (1994) *Current Biol 4(6):* 518-521. (**2**) Donahue BA, *et al.* (1994) *PNAS 91:* 8502-8506. (**3**) Evans MK, *et al.* (1993) *Cancer Res 53:* 5377-5381. (**4**) Ford JM, *et al.* (1994) *Molec Carcinogen 10:* 105-109. (**5**) Dumaz N, *et al.* (1993) *PNAS 90:* 10529-10533.

A5-003 STRAND-SPECIFIC AND SITE SPECIFIC RATES OF REPAIR OF BENZO(a)PYRENE-DIOL EPOXIDE (BPDE) ADDUCTS IN THE *HPRT* GENE OF HUMAN FIBROBLASTS, Veronica M. Maher, Ruey Hwa Chen, Dong Wei, and J. Justin McCormick, Carcinogenesis Laboratory, The Cancer Center, Michigan State University, East Lansing, MI 48824.

BPDE binds covalently to the N^2 position of guanine. To investigate the effect of excision repair on the kinds and location of mutations induced in human fibroblasts by BPDE, we synchronized cells, exposed them at the beginning of S-phase just when the target gene, *HPRT*, is replicated or in early G_1-phase to allow at least 12 hr for repair before the onset of replication. The frequency of mutants was 300 X 10^{-6} for cells treated in early S and 100 X 10^{-6} for cells given time for repair. 19/20 of the base substitutions in S-treated cells and 19/19 of those from G_1-treated cells involved G·C bp, mainly G·C --> T·A, but the distribution of the premutagenic lesions differed significantly. For cells treated in S, 24% were located in the transcribed strand; for G_1-treated cells, none were. No such cell cycle-dependent difference in frequency or distribution of mutations was seen in repair-deficient XP cells. These results suggested strand-specific repair of BPDE-induced damage. We tested this using UvrABC excinuclease and Southern blotting with strand-specific *HPRT* probes. Within 7 hr after treatment with 1.2 μM BPDE, 55% of the adducts had been removed from the transcribed strand, but only 25% from the nontranscribed strand; after 20 hr, these values were 87% and 58%. Only 38% had been removed from the genome overall. Mutation analysis also showed that in cells treated in S, 5% of the substitutions were at nt 212 and 5% at nt 229 in exon 3. However, in cells treated in early G_1 and allowed to repair, 21% were found at nt 212 and 10% at nt 229. No such difference in distribution was seen in XP cells, suggesting that repair at these sites was inefficient, i.e., that site-specific repair occurred. To test this, we adapted LM-PCR to measure the rate of removal of adducts from individual sites in exon 3. Cells were treated in G_1 with 0.5 μM BPDE and harvested at 0, 10, 20, 30 hr, and the DNA was analyzed for adducts using UvrABC. A gene-specific primer was annealed and extended to generate a blunt end at the site of each cut. A linker was ligated to the blunt end, and the desired fragments were isolated, amplified, and analyzed. The data showed that the rate of repair at individual sites varied and was slowest at nt 212 and 229. These results indicate that DNA repair plays an important role in determining the frequency and spectrum of mutations, and support the hypothesis that inefficient repair is involved in mutation hot spots. (NCI Grants CA21253 and CA56796).

Gene Induction in Response to Stress

A5-004 GENE INDUCTION PROFILES IN HUMAN AND BACTERIAL CELLS EXPOSED TO STRESS, Mark B. Benjamin[1], Cindy S. Orser[1], Marqué D. Todd[1], Pauline Gee[1], and Spencer B. Farr[1,2], [1]Xenometrix Inc. Boulder, Colorado and [2]Harvard School of Public Health, Boston, Massachusetts.

Cells respond in measurable ways to changes in their environment, such as heat, pH, and a wide range of both toxic and non-toxic stimuli. Many of these responses are mediated by the induction of transcription of a number of specific genes, whose transient and often rapid transcriptional activity is known as a "stress" response. Although the prototypical, and best characterized stress response is that of heat shock, there are now numerous bacterial and mammalian genes whose induction is indicative of a wide variety of cellular stresses. We have developed a battery of *Escherichia coli* and mammalian stress gene-reporter constructs and stably integrated them into bacterial and human cell strains and lines. Sixteen stress promoter:*lacZ* fusions were created and single site, single copy integrants generated in the *E.coli* genome to create independent bacterial strains. After exposing cells for 90 minutes to compounds soluble in either ethanol, water or dimethyl sulfoxide, the bacteria are lysed and *lacZ* expression monitored by the turnover of the substrate analog *o*-nitrophenyl-β-D-galactoside to yield intensely yellow *o*-nitrophenol. The amount of β-galactosidase activity is calculated and the fold induction for each gene over baseline is determined. These 16 different genes include those responsive to DNA damage (helicity changes as well as DNA modifications), protein perturbation, metals, and oxidative stress. Fourteen mammalian stress gene:reporter fusions have been constructed and independent multicopy integrant lines have been established using the human hepatoma cell line hepG2 as host. These fusions incorporate the chloramphenicol acetyltransferase (CAT) structural gene as a reporter. After cells have been exposed for 4-48 hours to compounds of interest, the cells are lysed and CAT protein is quantified by a standard sandwich ELISA. Promoters from genes responsive to DNA damage, protein perturbation, mitogenesis, aromatic hydrocarbons, metals, and retinoids have been converted into fusions in this assay format. Again, the fold induction of these mammalian genes is calculated over baseline. Induction "profiles" of cells generated with these assay systems show the immediate utility of these data: compounds of similar structure and activity generate similar induction profiles which reveal subtle differences in their mode of action or potency. The rapid nature of these assays (both can be completed in triplicate at ≥ 5 doses within 24 hours) will provide significant amounts of information regarding the mode of action of novel compounds. Using these induction profiles, closely related compounds may be ranked with respect to a number of toxicological and mechanistic endpoints.

A5-005 SIGNALING PATHWAYS INVOLVED IN TISSUE-SPECIFIC RESPONSES TO DIOXIN. William F. Greenlee, Yong Ming Tang, and Yu-Yuan Wo, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907-1334.

Many of the toxic responses elicited by dioxin and related compounds result at least in part from modulation of gene expression mediated by activation of the intracellular *Ah* receptor. Multiple gene regulatory mechanisms have been documented. These include: direct transcriptional activation (CYP1A1), mRNA stabilization (TGF-α), and transcriptional suppression (TGF-β_2) (1). Using a differential hybridization screening approach, we isolated four dioxin-responsive cDNAs from a human keratinocyte cell line developed as a model for dioxin-induced differentiation (2). Two of the cDNA clones represented genes encoding proteins involved in acute inflammatory responses and growth regulation: plasminogen activator inhibitor-2 (PAI-2), a regulator of the extracellular matrix proteolysis; and interleukin-1β, a cytokine. A third clone has been shown to be the first member of the cytochrome P450 1B sub-family (3). A 12 kb genomic clone containing the CYP1B1 gene was isolated from a human lung fibroblast genomic library. The CYP1B1 gene contains three exons and two introns and maps to human chromosome 2 at 2p21-22. The putative open reading frame (ORF) starts in the second exon and is 1632 bp in length. Nine potential dioxin-responsive enhancer (DRE) binding motifs are located within a 2.5 kb genomic fragment 5'-ward of the transcription initiation start site (TIS). In contrast to CYP1A1, CYP1B1 lacks a consensus TATA box in the promoter. Functional mapping of the CYP1B1 promoter indicates that the minimal promoter is located between -47 and +25 and contains a TATA-like sequence (TTAAAA) at -27. Enhanced promoter activity requires two SP1 sites located at -68 and -84. An initiator (Inr) sequence, TTGACTCT, is located between -3 to +5 and is required for maintaining a single start site of transcription, as judged by the appearance of multiple bands in in vitro transcription studies of promoter constructs containing a mutated Inr. Initial studies comparing rates of gene transcription with the level of mRNA transcripts for CYP1B1 and PAI-2 support the involvement of multiple dioxin-dependent regulatory mechanisms that include direct transcriptional activation, interaction with other transcription factors, and altered mRNA turnover.

(1) K Gaido, S Maness, and WF Greenlee (1992) J. Biol. Chem. 267:24591; (2) TR Sutter, K Guzman, KM Dold and WF Greenlee (1991) Science 254:415; (3) TR Sutter, YM Tang, CL Hayes, YYP Wo, EW Jabs, X Li, H Yin, CW Cody and WF Greenlee (1994) J. Biol. Chem 269:13092.

Receptor Mediated Toxicity

A5-006 ROLE OF THE MURINE ***[Ah]*** GENE BATTERY IN OXIDATIVE STRESS, Daniel W. Nebert, H.-C. Leonard Liang, Steven F. Reuter, Ross A. McKinnon, Howard G. Shertzer, Alvaro Puga and Vasilis Vasiliou, Department of Environmental Health, University of Cincinnati Medical Center, P.O. Box 670056, Cincinnati, Ohio.

We have examined the interrelationship of six genes in the mouse dioxin-inducible aromatic hydrocarbon-responsive ***[Ah]*** gene battery: two Phase I genes, ***Cyp1a1*** and ***Cyp1a2***; and four Phase II genes, ***Nmo1*** [an NAD(P)H:menadione oxidoreductase], ***Ahd4*** (ALDH3c; a cytosolic aldehyde dehydrogenase), ***Ugt1*06*** (a UDP glucuronosyltransferase), and ***Gsta1*** (Ya; a glutathione transferase)--in the hepatoma Hepa-1 wild-type (***wt***) cell line and its mutant derivatives, P_1^-(CYP1A1 metabolism-deficient), r^-(receptorless), and nt^-[Ah receptor nuclear translocation (ARNT)-impaired], and in several inbred and congenic mouse lines. In the ***wt*** cells, as in intact mice, transcription of the genes is up-regulated via the Ah receptor (AHR) bound to foreign ligand inducers such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) and benzo[a]pyrene. One or more aromatic hydrocarbon-responsive elements (AhREs), to which the dioxin-AHR-ARNT complex binds, exist in the 5' regulatory domain of all six mouse ***[Ah]*** genes. The 5' regulatory regions of all murine ***[Ah]*** Phase II genes, but not the ***[Ah]*** Phase I genes, contain an AP-1-like DNA motif called the electrophile responsive element (EpRE). A functional CYP1A1 or 1A2 protein appears to be necessary for the down-regulation of ***CYP1A1*** transcription (negative autoregulatory loop), as well as all four ***[Ah]*** Phase II genes, by way of a proposed negative response element (NRE); in fact, it is this criterion which qualifies a gene to be a member of the ***[Ah]*** battery (***i.e.***, other genes are dioxin- or electrophile-inducible, but not down-regulated by the CYP1A1 or 1A2 enzyme. There is an association between elevations in CYP1A1 and/or CYP1A2 activity and increases in oxidative stress. This laboratory has characterized a chromosome 7-mediated EpRE-dependent induction of the ***[Ah]*** Phase II genes, which elicits a differential effect of TCDD-inducibility on the ***[Ah]*** Phase I genes. This "oxidative stress response" (which also includes induction of Cu,Zn-SOD, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase) was first believed to reflect the absence of a putative repressor gene, located in the Chr 7 deletion (3,800 kb) of the homozygous ***14CoS/14CoS*** mouse, but, in studies with homozygous "knockout" mice, it now appears to be due to loss of the fumarylacetoacetate hydrolase (***Fah***) gene and absence of the enzyme (FAH), thereby resulting in elevated levels of very potent endogenous tyrosine oxidative electrophilic metabolites.

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Apoptosis

A5-007 APOPTOSIS AND THE CELL CYCLE, William Meikrantz and Robert Schlegel, Department of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA 02115 USA.

Apoptosis induced in HeLa cells by staurosporine was greatly potentiated by S phase arrest. In hydroxyurea (HU)-arrested cells, staurosporine was effective at doses as low as 1 ng/ml, with a maximum response following 8 h of exposure to 20 ng/ml. Other S phase-arrest agents (aphidicolin, high thymidine) were equally effective, whereas arrest early in G1 with lovastatin did not potentiate apoptosis. Since staurosporine is known to induce premature mitosis in S phase-arrested hamster cell lines, we tested the ability of other mitosis-inducing agents to promote apoptosis in HeLa cells. Caffeine, 6-dimethylaminopurine and okadaic acid all induced apoptosis in HeLa cells, but only following HU arrest. HU arrest also enabled γ radiation and TNF-α to induce apoptosis in HeLa cells, cells which are normally resistant to these two agents. Thus, the apoptosis-potentiating ability of S phase arrest is not restricted to inducers of premature mitosis. Radiation-induced apoptosis occurred without induction of p53 protein, which was present in asynchronously growing or S phase-arrested HeLa cells at virtually undetectable levels. We suggest that HU substitutes for the arrest that would normally be imposed by p53 via p21/WAF1. The effectiveness of all agents was drastically reduced in HeLa cell lines stably overexpressing the anti-apoptosis oncogene, *bcl-2* (≤2%, vs. 20-70% in *neo*-transfected controls). In each case tested, appearance of the condensed chromatin characteristic of apoptosis was accompanied by 2- to 7-fold increases in cyclin A-associated histone H1 kinase activity. Where examined, this was due to activation of both Cdc2 and Cdk2. Cyclin B-associated activity was minimal, remaining at <1% of its mitotic value, while cyclin E-associated activity was largely unaffected. Transient overexpression of the dominant-negative mutants Cdk2-dn and Cdc2-dn, which compete with the endogenous proteins for binding to cyclin A, significantly reduced the apoptotic response to TNF-α. Cdk5-dn had no effect. Susceptibility to TNF-α was unimpaired if Cdc2-dn and Cdk2-dn were co-transfected with cyclin A or wild type Cdk2; wild type Cdc2 was much less effective in blocking dominant negative function. This suggests that in HeLa cells it is primarily the cyclin A-Cdk2 complex which mediates apoptosis. Conversely, transient overexpression of Cdk2 in Bcl-2$^+$ HeLa cell lines led to a significant increase in apoptosis following treatment with HU + staurosporine, as compared with sham-transfected controls. These results clearly place Cdks on the biochemical pathway of apoptotic chromatin condensation. Interestingly, stable overexpression of *bcl-2* reduced the accumulation of Cdc2 and Cdk2 in the nucleus during S phase arrest without affecting the nuclear localization of cyclin A, cyclin E or Myc. Reduced amounts of nuclear Cdks were paralleled by an increased proportion of cells in G1, an increased cell size, and a slightly slower doubling time in Bcl-2$^+$ cells. This suggests that Bcl-2 may play a "gate-keeper" role in the cell, restricting access to the cell nucleus of proteins required for both cell division and cell death.

Molecular Mechanisms of Carcinogenesis

A5-008 INDUCTION BY GENOTOXIC STRESS OF MAMMALIAN GENES ASSOCIATED WITH GROWTH-ARREST AND APOPTOSIS, AND THE ROLE FOR THE P53 TUMOR SUPPRESSOR IN THEIR REGULATION. Albert J. Fornace, Jr., NCI, Bethesda, MD.

The gadd (growth-arrest and DNA-damage inducible) genes represent a group of often coordinately-expressed genes that are induced by genotoxic stress and certain other growth-arrest signals. These genes are rapidly induced by agents producing high levels of DNA base damage, such as UV radiation and the alkylating agent MMS (methylmethane sulfonate), in many mammalian cells; in addition, they are also strongly but more slowly induced by medium depletion (starvation) (designated "MD"). Induction of *GADD45* by ionizing radiation (IR), as well as activation of the G_1 checkpoint by IR, is dependent on *wt* p53[1], which has subsequently been found to also be the case for *MDM2* and *WAF1/CIP1*. In some myeloid and lymphoid cells, IR rapidly induced apoptosis and also induced *GADD153* and *GADD34*; interestingly, *GADD45* and the apoptosis-promoting gene *BAX* were only strongly induced in such cells with wt p53 status. Thus, a spectrum of growth-arrest and apoptosis associated genes are induced following IR in cells dependent on their p53 status and onset of apoptosis. Unlike the case for IR induction of *GADD45*, the other gadd genes, and *WAF1/CIP1* is not strictly dependent on p53 after treatment with UV, MMS, or MD. These findings indicate the presence of 2 response mechanisms for *GADD45* – the IR-type and the UV/MMS/MD-type. Surprisingly, induction of p53 activity, as measured by a variety of approaches, was much stronger for UV, MMS or MD in p53 wt cells compared to IR. In addition, the spectrum of inducing agents for *GADD45* and the other gadd genes is very similar to that for p53 activation. Current studies indicate that in addition to the p53 requirement for induction of *GADD45* by IR, p53 can have a cooperative effect in the induction of the gadd genes by a variety of stresses. Evidence includes reduced responses in mutant cells, such as ataxia telangiectasia, and reduced induction with gadd-promoter CAT-reporter gene constructs in cells containing dominant-negative p53 expression vectors. Taken together, these findings and others (to be presented) indicate that p53 has a role in both of these response pathways: p53 is required for the *gadd45* IR response and can have a cooperative effect on the UV/MMS/MD pathway, but is not required.

While the functions of the Gadd and related proteins are uncertain, several common features of these proteins have been found. The protein products of 3 gadd genes plus a related gene *MyD118*, which is also growth-arrest and DNA-damage inducible, encode unusually acidic proteins with very similar charge characteristics[3]; this property is shared with another p53-regulated gene *MDM2*. The use of a short term transfection assay, where expression vectors for one or a combination of these gadd/MyD genes were transfected with a selectable marker into several different human tumor cell lines, provides direct evidence for the growth inhibitory functions of the products of these genes and their ability to cooperate in growth suppression. Studies on interactions of Gadd45 with other nuclear proteins, which may explain some of these effects, will also be presented. These genes probably represent the downstream effectors for multiple genotoxic stress responses and certain other cellular stress responses resulting in growth arrest and apoptosis.

1. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, Jr, A. J. 1992. A mammalian cell cycle checkpoint utilizing p53 and *GADD45* is defective in ataxia telangiectasia. Cell *71*, 587-597.

A5-009 ABROGATION OF CELL CYCLE CHECKPOINT CONTROL IN PRENEOPLASTIC CELLS, Thea D. Tlsty, Lineberger Comprehensive Cancer Center, Department of Pathology, and Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599-7295

Genomic integrity is maintained by a network of cellular activities that assesses the status of the genome at a given point in time and provides signals to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. We have used viral proteins involved in malignant transformation to investigate cellular pathways that may be perturbed during loss of genomic stability. Recent studies have indentified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling neoplasia. Among the targets of the viral oncoproteins are the products of the p53 and retinoblastoma (Rb) tumor suppressor genes. We demonstrate that the expression of human papillomavirus type 16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of the network of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins, including Rb, resulted in a p53-independent alteration in cell cycle control, massive cell death and polyploidy upon PALA treatment. These results demonstrate that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells.

Transgenic Models of Toxicity

A5-010 THE EFFECTS OF INCREASED EXPRESSION OF CuZn-SUPEROXIDE DISMUTASE IN TRANSGENIC MICE. Charles J. Epstein[1], Ting-Ting Huang[1], Elaine Carlson[1], Pak H. Chan[1], John P. Phillips[2], and Jean L. Cadet[3], [1]University of California, San Francisco, CA 94143, [2]University of Guelph, Guelph, Ontario N1G 2W1, and [3]NIH/NIDA Addiction Research Center, Baltimore, MD 21224.

The superoxide dismutases, CuZnSOD and MnSOD, which convert superoxide anions to hydrogen peroxide, are the first of a series of enzymes that mediate the metabolism of potentially deleterious oxygen free radicals. However, their roles in a variety of situations in which oxidative stress is believed to be of importance have not been delineated. Transgenic mouse technology permits the systematic alteration of individual components of the free radical detoxification scheme, and we have employed a series of mice transgenic for CuZnSOD for this purpose. Heterozygous transgenic mice carrying between 2 and 8 copies of the human genomic sequence for CuZnSOD under control of the native promoter express between 1.8 and 3.1 times the nontransgenic level of CuZnSOD in erythrocytes, fibroblasts, and neurons. High expressing homozygous transgenic animals have 5 times control levels. Transgenic mice with 3 to 5 times increased CuZnSOD are protected to various degrees in vivo against a variety of acute and chronic insults to the central nervous system in which oxygen free radicals (O_2^-) and/or nitric oxide (NO·) are believed to play a pathogenic role. These insults, both physical and chemical in nature, include cold injury, blunt trauma, ischemia and reperfusion (stroke), and the toxic effects on dopaminergic neurons of MPTP, methamphetamine, and methamphetamine derivatives (which produce models of Parkinson disease). The transgenic mice are also more resistant to the induction of type I diabetes mellitus by the pancreatic islet ß-cell toxins, alloxan and streptozotocin. These findings indicate that relatively small changes in CuZnSOD activity can have profound effects on the cellular response to a variety of stimuli that cause damage through the generation of oxygen free radicals. As a result, CuZnSOD transgenic mice serve as useful probes for investigating the potential roles of such radicals in pathological processes initiated by a wide range of toxic agents and processes.

Human in vivo Toxicity Testing

A5-011 IN VIVO SOMATIC MUTATIONS IN HUMANS REFLECT ENDOGENOUS AND EXOGENOUS GENETIC DAMAGE, Richard J. Albertini, Janice A. Nicklas, and J. Patrick O'Neill, Vermont Cancer Center, University of Vermont, Burlington.

Genetic damage arising *in vivo* in human somatic cells is reflected as mutations in reporter genes such as *hypoxanthine-guanine phosphoribosyltransferase (hprt)*. The background mutant frequency (Mf) for normal young adults, determined in peripheral blood T-lymphocytes, is 5-10 x 10^{-6}, indicating the endogenous and ubiquitous exogenous genotoxicity against which specific environmental exposures must be measured. More precisely, there is a pronounced age effect on background Mf described by **$\ln Mf = 0.38 - 1.77\ CE + 0.67 \ln (Age + 1)$** [where CE is non-selected cloning efficiency]. This relationship is the basis for determining residual Mfs reflecting specific exposures. At the molecular level, approximately 15% of adult background *hprt* mutations show gross structural alterations, e.g. large deletions from approximately 200 bp to 3.5 Mb, while 85% have base substitutions in coding sequences, splice site alterations, frameshifts, smaller deletions, with a predominance of base substitutions in GC base pairs. Two characteristics of the background reflect endogenous genetic damage. First, *in vivo* clonality among mutant as compared to non-mutant isolates indicates the important role of cell division in mutation. Second, the most profound *in vivo* mutagenic influence thus far observed in human T-cells is that resulting from illegitimate V(D)J recombinase activity in the fetus and during childhood, where 40% or more of newborn *hprt* mutations have intragenic deletions mediated by this enzyme complex. (Illegitimate V(D)J recombinase mediated deletions/rearrangements also characterize lymphoid malignancies when they occur in critical genetic regions.) Also indicative of endogenous influences on *in vivo* mutations is their occurrence at increased frequencies in individuals with DNA repair defects. Exogenous genetic damage at *hprt* is reflected as increased Mf values under conditions of specific environmental exposures, e.g. tobacco smoke, ionizing irradiations from several sources, cytotoxic chemotherapies, and specific occupational and household mutagen exposures. Some elevations have been correlated with specific DNA adduct levels and changes in *hprt* mutational spectrum. Fetal mutations determined in newborns delivered to women from a lower socioeconomic status showed fewer V(D)J recombinase mediated and more "point mutations," suggesting that the latter reflect the environmental effect. Cancer patients receiving a form of total body irradiation show a far greater frequency of *hprt* deletions. Data have been presented to suggest a specific point mutation in ethylene oxide exposure. Therefore, both endogenous and exogenous genetic damage is detected in *in vivo hprt* mutations. It remains to be determined whether those that reproduce mutagenic processes seen in cancer are of greater relevance as biomarkers of genotoxic effect and predictive value for individual cancer risk. (Research supported by NCI RO1 CA30688 and DOE FG028760502. NCI and DOE support does not constitute an endorsement of the views expressed.)

A5-012 NICKEL CARCINOGENESIS: INACTIVATION OF SENESCENCE AND OTHER CANCER-RELATED GENES BY ENHANCED HETEROCHROMATIN CONDENSATION AND DNA HYPERMETHYLATION, Max Costa, Department of Environmental Medicine and Kaplan Comprehensive Cancer Center, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Certain particulate nickel compounds, i.e., crystalline Ni_3S_2, are well-established human carcinogens and induce tumors in animals at virtually any site of administration. Carcinogenic nickel compounds generate oxygen radicals in cells; however, these do not have mutagenic consequences because of the selective interaction of nickel(II) with genetically inactive heterochromatin and the caged generation of oxygen radicals at this site. The avid binding of nickel to core histones and to H_1 as well as the general high affinity that Ni^{+2} has for the proteins compared to DNA drives the selective binding of Ni^{+2} to heterochromatin. We have previously reported that nickel-induced transformation of male Chinese hamster embryo cells was associated with inactivation of a senescence gene on the highly heterochromatic X chromosome by DNA hypermethylation (*Science* **251**:796, 1991). It was also found that the thrombospondin gene was inactivated in nickel-transformed cells by loss of transcription factors that positively regulate its promoter (*Mol. Cell Biol.* **14**:1851, 1994). Nickel-induced DNA methylation was implicated in the loss of expression of these transcription factors. Bacterial *gpt* transgenes inserted into V79 cells have yielded one particular cell line that was very responsive to nickel-induced 6TG resistance. However, the incidence of this resistance induced by nickel was too high for it to have been a mutagenic event (10^3). Subsequent studies revealed that the *gpt* gene was inactivated by nickel-induced increased chromatin condensation and hypermethylation of the 3' and 5' flanking as well as the coding regions of this gene. This gene was located near a dense heterochromatic region of chromosome 1. Additionally, Ni-induced 6TG resistance cell types were readily reverted to wild type with the drug azacytidine which induces hypomethylation of DNA. Nickel induced 6TG resistant cells exhibited enhanced chromatin condensation around the *gpt* gene as evidenced by DNaseI sensitivity studies. Carcinogenic nickel compounds exhibited low activity in inducing 6TG resistance in other transgenic cell lines where the *gpt* gene was inserted in euchromatin. Chromatin fractionation studies demonstrated that the *gpt* gene was found in a biochemically distinct heterochromatin fraction in G12 cells and much less of the gene was associated with the heterochromatin in non-responsive transgenic cell lines. However, when cells became resistant to 6TG following nickel exposure, the gene was found to a much greater extent in the heterochromatin fraction. A model emerges from these studies with regard to how nickel might be producing the inactivation of genes that maintain a "normal" cell. Nickel has previously been shown to increase chromatin condensation by substituting for Mg^{2+} and excess Mg^{2+} inhibits both nickel induced damage to heterochromatin and nickel carcinogenesis. Ni^{2+} binds to heterochromatin causing the extension of heterochromatin into neighboring euchromatin resulting in genes that are active in euchromatin to become incorporated into heterochromatin. This can be visualized as a spool with thread where the inactive heterochromatin DNA is on the spool while the active euchromatic DNA is in the thread coming off the spool. Nickel causes more thread to be rolled up on the spool and thus initially genetically active DNA is inactivated by an increased condensed state. The newly condensed DNA is now methylated as a result of its presence in heterochromatin (on the spool), and since DNA methylation patterns are faithfully copied after DNA replication, this DNA will now be inherited in an inactive state in all daughter cells.

A5-013 MOLECULAR EPIDEMIOLOGY IN ENVIRONMENTAL CARCINOGENESIS. Frederica P. Perera, Columbia University School of Public Health, Division of Environmental Sciences, 60 Haven Ave., B-109, NY NY 10032.

Molecular epidemiology aims at cancer prevention by incorporating laboratory methods to document the molecular dose and preclinical effects of carcinogens as well as factors that increase individual susceptibility to carcinogens. It has significant potential in preventing cancer due to environmental exposures (related to lifestyle, occupation or ambient pollution). This presentation will give examples of our recent validation studies of biologic markers such as carcinogen-DNA and -protein adducts, gene and chromosomal mutation, alterations in target oncogenes or tumor suppressor genes, polymorphisms in putative "susceptibility" genes (individual P450s, glutathione transferase M1) and serum levels of micronutrients[1]. This research involves adults, infants and children exposed to varying levels of carcinogens, as well as cancer cases and controls. Overall, dose-response relationships have been seen between various biomarkers and environmental exposures such as polycyclic aromatic hydrocarbons (PAH), cigarette smoke (active and passive) and ambient, indoor and workplace air pollution. However, there is significant interindividual variation in biomarkers which appears to reflect a modulating effect on biomarkers (hence potential risk) by genetic and acquired susceptibility factors. Ongoing retrospective and nested case-control studies of lung and breast cancer are examining the association between biomarkers and cancer risk. These results are encouraging that, once validated, biomarkers can be useful in identifying populations and individuals at risk--in time to intervene effectively.

[1]See for example Perera et al. Nature 360:256-258, 1992; Perera et al. Carcinogenesis 14:969-973, 1993; and Crawford et al. JNCI 86:1398-1402, 1994.

A5-014 PROTEIN BIOMARKERS IN THE MOLECULAR DOSIMETRY OF TOXICANTS, Steven R. Tannenbaum, Department of Chemistry and Division of Toxicology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Xenobiotic compounds that react with DNA also react with nucleophilic centers on proteins. On this basis, such adducts have been proposed as surrogates for DNA adducts and this approach has been successfully employed in a variety of molecular epidemiological studies. This paper will review the chemistry of adduct formation in relation to the structure of the xenobiotic compound and the selective nature of proteins which directs adducts to specific side-chains. Methods for quantitative analysis of adducts and approaches to detection of unknown adducts will also be discussed.

Our research to date has encompassed a wide variety of compounds known or suspected to cause cancer in people: aromatic amines, heterocyclic amines, polycyclic aromatic hydrocarbons, aflatoxins, and assorted alkylating agents. The most advanced of these problems is that of aromatic amines in which several different types of studies on human populations have already been completed using hemoglobin adducts of 4-aminobiphenyl as the biomarker. This approach has paid major dividends in the analysis of human polymorphisms in carcinogen metabolism.

Recently we have focussed on histones as long term biomarkers of carcinogen exposure. These proteins appear to have unique reactive sites for a variety of carcinogens. Their proximity to nuclear DNA combined with their tissue specificity and stability offer potentially unique opportunities in future molecular epidemiological studies.

This work was supported by Grants No. ES05622 and ES04675 from the National Institutes of Health.

In vitro Toxicity Testing

A5-015 MOLECULAR BIOMARKERS OF MUTAGENESIS AND CARCINOGENESIS, Pauline Gee[1,2], Dorothy Maron[2], Marcus Trione[2], Christopher Sommers[1], Henry Cobb[1], Anatoly Portnoy[1], Spencer Farr[1], and Bruce Ames[2], [1]Xenometrix Inc., Boulder, CO 80301 and [2]University of California, Berkeley, CA 94720.

While it is extremely difficult to predict the mutagenic and carcinogenic potential of many compounds, *in vitro* testing of these compounds often reveal molecular mechanisms that are valid *in vivo*. However, *in vitro* tests that measure mutgenicity in microbial and mammalian cell systems have performed somewhat inadequately in correlation to tumor formation in whole animal tests that are routinely used to predict effects in humans. There are many parameters that determine one's predisposition to tumor formation such as the stage of development at the time of exposure, genetic makeup and differences in lifestyle. Such parameters are not measurable by *in vitro* systems. Nevertheless, *in vitro* testing systems that measure appropriate endpoints which indicate underlying mechanisms of toxicity provide information on cellular responses to structural moeities of the compound or a mixture of different compounds. Damage to DNA often results in premutagenic lesions which are detectable in short term tests like the *Salmonella* mutagenicity assay. A set of strains that indicates the type of base substitution upon detection corroborate the bases at which premutagenic lesions are formed. For example, adducts of the psoralen, angelicin, when activated by long wavelength UV light are formed preferentially at pyrimidines. The relative proportion of pyrimidine adducts at T•A basepairs is greater than at C•G basepairs. Therefore these proportions should be and were reflected in the reversion spectrum obtained in this set of base-specific *Salmonella* strains. Other parameters that determine which pathways or the degree of reversion (or mutagenesis) were easily measured in bacterial systems but the relevance of this type of information to whole animals (including humans) is yet to be determined. These parameters might include the level of gene expression of the DNA target and/or the context of the susceptible base. We found that an increase in the expression of the gene containing the target basepair enhanced the sensitivity of the target to revert or mutate by different reversion pathways. This effect depended on the target basepair as well as the DNA sequence immediately adjacent to the target basepair. Thus molecular information like mutational spectra of a compound, or the level of expression of a gene can be easily gathered by *in vitro* systems. If these parameters are central to the mechanisms of action of the genotoxin, they pose pertinent questions to answer in *in vivo* systems. The validity of mechanistic models based on information at the molecular level should be tested in the appropriate animal. The *in vitro* endpoints can be then be interpreted in the context of whole body metabolism and can be used as molecular biomarkers of mutagenesis and/or carcinogenesis and be extremely valuable to predict effects in humans.

Molecular Epidemiology

A5-016 CANCER PREVENTION: WHAT ASSAYS ARE NEEDED?, Bruce N. Ames, Division of Biochemistry and Molecular Biology, University of California, Berkeley, Berkeley, CA 94720

Aging appears to be in good part due to the oxidants produced as by-products of normal metabolism. These oxidants are the same mutagens that are produced by radiation, and cause damage to DNA, proteins, and lipids. The DNA in each cell of a normal rat receives on average about 100,000 oxidative lesions per day. DNA repair enzymes constantly remove this damage, but do not keep up: an old rat has about 2 million oxidative lesions in the DNA of each cell.

The degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction, are increasingly found to have, in good part, an oxidative origin. It is argued that dietary antioxidants, such as Vitamins C and E and carotenoids, play a major role in minimizing this damage and that most of the world's population is receiving inadequate amounts of them, at a great cost to health. The main source of dietary antioxidants is fruits and vegetables. Humans should eat five portions of fruits and vegetables per day, yet only 9% of the U.S. population eats that much. Epidemiological studies show that the incidence of most types of cancer is double among people who eat few fruits and vegetables as compared to those who eat about five portions per day.

The three main causes of cancer are smoking, dietary imbalances (excess fat and calories; inadequate intake of fruits, vegetables, fiber, and calcium), and chronic infections leading to chronic inflammation (hepatitis B and C viruses, *Helicobacter pylori* infection, schistosomiasis, etc.) [1]. Chronic inflammation is a major cause of cancer in the world because it releases powerful oxidants which both stimulate cell division and are mutagens. Past occupational exposures might cause about 2% of current human cancer, a major part being asbestos exposure in smokers, and industrial or synthetic chemical pollution causes less than 1%, in my view.

Lesions in DNA are formed when DNA is damaged and cell division converts DNA lesions to mutations. Agents increasing either lesions or cell division increase mutations and as a consequence increase cancer incidence. Hormones stimulating cell division increase cancer incidence (e.g., levels of estrogen in breast cancer).

Improved assays in humans are needed for assaying damage to proteins, lipids, and DNA; for inflammatory damage by nitration from peroxynitrite and chlorination by hypochlorite and for oxidative damage.

[Ames, B.N., Shigenaga, M.K., and Hagen, T.M. (1993), Oxidants, Antioxidants, and the Degenerative Diseases of Aging. *Proc. Natl. Acad. Sci. USA* 90, 7915-7922.]

A5-017 ROLE OF XENOBIOTIC-METABOLIZING ENZYMES IN CANCER SUSCEPTIBILITY, Frank J. Gonzalez, Laboratory of Molecular Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

It has long been recognized that exposure to environmental and dietary chemicals are likely to be responsible for the vast majority of human cancers. A large number of enzymes participate in both activation and inactivation pathways of carcinogen metabolism. These enzymes mainly function in xenobiotic metabolism and are not thought to play critical roles in metabolism of endogenous chemicals, such as hormones. A high degree of interindividual variability exists in levels of expression of xenobiotic-metabolizing enzymes and some of this variability is due to genetic polymorphism. The critical roles of these enzymes in carcinogen metabolism and this genetic variability in their expression lead to the possibility that xenobiotic enzyme polymorphisms are associated with cancer risk. This risk would be manifest with environmental or dietary exposure to carcinogens. Cytochromes P450 are the key enzymes required for the metabolic activation of procarcinogens. A large number of P450s exist but a select few have been shown to metabolize various classes of known human and rodent carcinogens. CYP1A1 and CYP1B1 activate polycyclic aromatic hydrocarbons. CYP1A2 activates aflatoxin B1, arylamines and heterocyclic amines. CYP2A6 and CYP2E1 activate low molecular weight nitrosamines and tobacco-specific nitrosamines and CYP2E1 metabolizes a large number of low molecular weight cancer suspect agents. CYP3A4 and CYP3A5 also activate aflatoxins. The phase II conjugating enzymes function primarily in neutralizing active metabolites but in some cases participate in metabolic activation pathways for arylamines, heterocyclic amines, and vinyl halides. A number of genetic polymorphisms in P450s and transferase enzymes have been described, along with phenotyping and genotyping methods for use in their detection. There has also been progress in developing methods to identify DNA and protein adducts that serve as biomarkers for both carcinogen exposure and expression of the activating enzymes. The substrate specificities and polymorphisms of human P450s will be reviewed. The results of case control studies will be presented that demonstrate associations of polymorphic genotypes and phenotypes of P450s and transferases with cancer risk.

A5-018 CAFFEINE METABOLISM AS A MEASURE OF CYTOCHROME P450 FUNCTION, Bing-Kou Tang, Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

We chose to investigate caffeine (137X) as a probe for the assessment of variability in drug biotransformation capacity in human populations for its safety and its ubiquitous use. The caffeine-based methods have indicated high CYP1A2 values in cigarette smokers, in children, and in subjects exposed to polybrominated biphenyls or to Omeprazol. CYP1A2 is low in subjects on low-protein diets, in patients with various liver disease, in women taking contraceptive medication, and in women who are pregnant.

Several caffeine-based methods were used for the determination of CYP1A2 activity. (1) Measurement of systemic caffeine clearance may serve as a standard of convenience since the total involvement of CYP1A2 in caffeine metabolism accounts for more than 95 % of caffeine plasma or blood clearance. (2) The caffeine breath test which requires labelled caffeine and a specialized equipment has demonstrated convincingly its utility as measurement of CYP1A2 activity. (3) Two caffeine-based urinary methods have been proposed and compared i.e. the CMR, the caffeine metabolite ratio, (AFMU+1X+1U)/17U, and the PCUR method, the paraxanthine caffeine urinary ratio, (17X+17U)/137X.

Recently, a caffeine test has been proposed as specific marker of cytochrome P450 2E1, the ethanol inducible cytochrome (CYP2E1). This is based on the discovery of selective participation of CYP2E1 in caffeine biotransformation and the discovery of a shift of caffeine metabolite profiles in urine of alcoholics.

Therefore, the caffeine test, once validated for CYP2E1, will become a single test for monitoring multiple liver enzymes identified in carcinogen activation, i.e. CYP1A2, CYP2E1, the polymorphic N-acetyltransferase (NAT2), and xanthine oxidase. Greatly reduced costs of future toxicological and epidemiological monitoring can be anticipated. (Supported by Medical Research Council of Canada, NIH and NIAAA).

Late Abstracts

THE TRANSCRIPTIONAL RESPONSE TO UVC, TO H_2O_2, AND TO ALKYLATING AGENTS, Axel Knebel, Dagmar Wilhelm, Hans van Dam, Christoph Sachsenmaier, Andrea Kolbus, Christine Blattner, Hans J. Rahmsdorf, Peter Angel, and Peter Herrlich, Kernforschungszentrum Karlsruhe, Institut für Genetik, Postfach 3640, D-76021 Karlsruhe, Germany.

The transcriptional response to genotoxic agents serves protective purposes. How this is achieved is yet ill-understood. Part of the protection seems to be established by a c-Jun dependent genetic program since cells from Jun null mice are very UV sensitive (Schreiber & Wagner, unpublished). Jun dependent gene expression depends on signalling from outside the cell. Interestingly inhibition of signalling by blocking tyrosine protein kinases (tyrphostin) also increased UV sensitivity (1). The induced transcription of c-jun and of other members of the Fos/Jun/ATF family as well as the signal transfer to preexisting Jun protein is likely to constitute a decisive first step in the relevant change of gene expression upon treatment of cells with genotoxic agents. The presentation will deal with the molecular steps involved in the response within the first 5 to 10 minutes: Ligand independent activation of growth factor receptors, activation of stress-activated protein kinases and their substrate specificity, phosphorylation of transcription factors.

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THE HEAT SHOCK RESPONSE: SENSING AND RESPONDING TO CHANGES IN THE ENVIRONMENT, Richard I. Morimoto, Jose Cotto, Michael Kline, Paul Kroeger, Betty Lee, Sanjeev Satyal, and Yanhong Shi, Department of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston, Il. 60208.

Common to all organisms is an essential, highly conserved and exquisitely regulated cellular response to sub-optimal physiological conditions. The activation of heat shock (HS) gene expression in response to heat shock, oxidative stress, heavy metals, various toxic chemicals, infection, and anti-inflammatory drugs, resulting in the elevated synthesis of a family of stress-induced or heat shock proteins (hsp's) affords the cell with a protective mechanism against acute exposure which if left unchecked, leads to irreversible cell damage and ultimately cell death. The stress-induced proteins, some of which are also known as molecular chaperones, have essential roles in protein biosynthesis, specifically in the transport, translocation and regulation of protein folding through their ability to recognize incompletely folded proteins. Exposure to stress leads to the transcriptional activation of HS genes through the activation of heat shock factor (HSF), the initial step in the inducible transcription of heat shock genes. Vertebrate cells ubiquitously express a family of HSF genes, however for each HSF, DNA binding activity is negatively regulated. HSF1, the predominant heat shock and stress-responsive factor is a monomer in the control cells and a trimer in the DNA binding state. The process by which HSF1 detects and responds to stress involves multiple steps including oligomerization, acquisition of DNA binding activity, translocation, and phosphorylation. Trimerization and acquisition of DNA binding can occur without inducible phosphorylation which suggests that phosphorylation of HSF1 may have a role in modulation or attenuation of the transcriptional response. During attenuation of the heat shock response, HSF1 associates with specific heat shock proteins providing a possible mechanism by which HSF1 activity is autoregulated during the recovery from stress.

INTERACTIONS BETWEEN THE ARYL HYDROCARBON AND ESTROGEN RECEPTOR-MEDIATED SIGNAL TRANSDUCTION PATHWAYS, Stephen Safe, Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) binds with high affinity to the aryl hydrocarbon (Ah) receptor and elicits a diverse spectrum of toxic and biochemical responses. TCDD and related Ah receptor agonists characteristically induce the expression of several genes including CYP1A1, CYP1A2, CYP1B1, glutathione S-transferase Ya, menadione oxidoreductase, aldehyde-3-dehydrogenase and glucuronosyl transferase (1). The induction response requires formation of a heterodimeric nuclear Ah receptor complex containing the liganded Ah receptor and Ah receptor nuclear translocation (Arnt) proteins. The Ah receptor complex acts as a *trans*-acting ligand-induced transcription factor which interacts with dioxin or xenobiotic responsive elements (DREs or XREs) located in 5'-flanking regions of inducible genes. The resulting protein-DNA interaction is required for transactivation of gene expression; however, it is apparent that many other cell-specific factors influence the induction response. Ah receptor agonists also inhibit expression of several genes and/or related activities including phosphoenol pyruvate carboxykinase, uroporphyrinogen decarboxylase, c-fos, and the epidermal growth factor receptor. TCDD also exhibits diverse antiestrogenic responses in the rodent uterus and mammary and in human breast cancer cell lines and recent studies in this laboratory have focused on characterizing the molecular mechanisms associated with TCDD-induced antiestrogenicity (2,3). 17β-Estradiol (E2) induces cathepsin D gene expression in MCF-7 human breast cancer cells; moreover, TCDD and related compounds inhibit E2-induced cathepsin D protein and mRNA levels and the rate of gene expression. Initial studies identified an estrogen receptor (ER)/Sp1-like sequence ($GGGCGG(n)_{23}ACGGG$) in the non-coding strand of the cathepsin D promoter (-199 to -165) which appears to be responsible for the E2-responsiveness of this gene. The E2-responsive ER/Sp1 sequence (-199 to -165) in the cathepsin D 5'-region contains an imperfect GCGCGTG (-175/-181) xenobiotic responsive element (XRE); the role of this sequence in Ah responsiveness was investigated in gel electrophoretic mobility shift assays and with plasmid constructs containing wild-type ER/Sp1 oligo or a mutant ER/Sp1-*"XRE"* oligo containing two C → A mutations in the XRE sequence (antisense strand). In plasmid constructs which contained a chloramphenicol acetyl transferase (CAT) reporter gene and the wild-type ER/Sp1 promoter sequence, E2-induced CAT activity and mRNA levels were inhibited by TCDD; in contrast, TCDD did not inhibit the E2-induced responses using the mutant ER/Sp1-*"XRE"* plasmids. Electrophoretic mobility shift assays showed that the nuclear or transformed cytosolic Ah receptor complex blocked formation of the ER/Sp1 complex using the wild-type but not the ER/Sp1 mutant oligo. These data demonstrate that Ah-receptor-mediated inhibition of E2-induced *cathepsin D* gene expression is due to disruption of the ER/Sp1 complex by direct interaction of the Ah receptor complex with a strategically located XRE. The role of the nuclear Ah receptor complex as an inhibitor of other E2-induced genes will also be discussed.

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Molecular Mechanisms of DNA Repair and Recombination; Gene Induction in Response to Stress

A5-100 ACTIVATION OF *GADD153* BY DNA DAMAGING AGENTS IN CISPLATIN RESISTANT AND PARENTAL HELA CELLS, Helen B. Eastman and Nikki J. Holbrook, Unit on Gene Expression and Aging, National Institute on Aging, Baltimore, MD 21224

Induction of the *GADD153* promoter in response to DNA damaging agents was examined in cisplatin (DDP) resistant (HeLaR1 & HeLaR3) and sensitive (HeLaC) cell lines. HeLaR1 and R3 cells have elevated levels of an XPE binding factor (a protein which binds to damaged DNA and is present in reduced amounts in repair deficient xeroderma pigmentosum (XP) cells from complementation group E) (Chu *et al.* PNAS 87: 3324, 1990). Using a colony formation assay we determined the ID_{50} for DDP and UV in these cell lines. HeLaR1 was 8-fold more resistant to DDP and 2-fold more resistant to UV and HeLaR3 was 13.6-fold more resistant to DDP and 3.4-fold more resistant to UV than the parental cell line HeLaC. The response of a transiently transfected *GADD153* promoter/CAT construct to DDP, UV and the DNA alkylating agent methyl methanesulfonate (MMS) was measured in these cell lines. The order of responsiveness to all 3 agents was HeLaC>>HeLaR1>HeLaR3, as shown below.

Fold Induction of *GADD153*/CAT

	DDP (15μg/ml)	UV (30 J/m^2)	MMS (100 μg/ml)
HeLaC	10	26	45
HeLaR1	4	8	10
HeLaR3	2	6	8

In summary, we have observed that HeLaR1 and R3 cells are less sensitive to the cytotoxic effects of various DNA damaging agents and that, in these cells, a *GADD153*/CAT construct is induced to a lesser extent by these agents. These findings suggest that damage resulting from treatment with these agents is directly involved in the signalling pathway leading to *GADD153* induction.

A5-101 YEAST RAD9 MUTANTS DEFECTIVE IN CELL CYCLE ARREST AT THE G_2-M CELL CYCLE CHECKPOINT EXHIBIT ELEVATED FREQUENCIES OF ECTOPIC RECOMBINATION IN RESPONSE TO GENOTOXIC AGENTS, Michael Fasullo and Joseph Koudelik, Department of Radiation Therapy, Loyola University Medical School, 2160 S. First Avenue, Maywood, IL 60153

DNA damaging agents can induce cell cycle arrest at cell cycle checkpoints and recombination; however, the functions of cell cycle arrest and in modulating stimulated, mitotic recombination have not been elucidated. *RAD9*$^+$ and *rad9*$^-$ congenic haploid and diploid strains were constructed that monitor both sister chromatid recombination and reciprocal translocations using the *his3* recombinational substrates. The spontaneous rate of mitotic, ectopic recombination is elevated ~10x in a homozygous *rad9* diploid from 1.8×10^{-7} to 1.5×10^{-6}. The number of stimulated His$^+$ recombinants is greater when the *rad9* diploid is exposed to γ-rays, UV, MMS, and 4NQO, as compared to the *RAD9*$^+$ diploid, and a six-fold difference in the number of stimulated recombinants has been observed after exposure to radiation. Interestingly, initiation of translocations by a single double-strand break in diploid strains is the same in *RAD9*+ as in *rad9* mutants. We hypothesize that the ability of DNA damaging to trigger both cell cycle arrest and sister chromatid exchange is one mechanism for the control of the initiation of ectopic recombination. However, in *rad9* mutant strains the stimulation of sister-chromatid recombination with either NQO or MMS is the same as in *RAD9*+ strains. We suggest that stimulation of sister-chromatid recombination by genotoxic agents does not depend upon the *RAD9* function.

A5-102 MECHANISMS OF HETEROCYCLIC AMINE TOXICITY IN CULTURED MAMMALIAN CELLS, James W. Gaubatz and Susan Rooks, Department of Biochemistry and Molecular Biology, University of South Alabama, Mobile, AL 36688

Heterocyclic amines (HCA) are produced during the cooking of meats and when properly activated, will form adducts with DNA. 2-Amino-3,8-dimethylimidazol[4,5-*f*]quinoxaline (MeIQx) is a HCA that is abundant in our diets, is highly mutagenic in bacteria, and induces tumors in rodents. MeIQx is activated by N-hydroxylation to N-OH-MeIQx. Recent work has indicated that some HCAs preferentially damage DNA in heart muscle cells, and that N-OH-MeIQx induced DNA adducts are inefficiently repaired in myocytes. Since adult cardiac myocytes are terminally differentiated cells that have lost their ability to divide, the capacity to repair DNA damage is a critical factor in the cardiomyocytes proper function and longevity. We have compared the relative toxicity of N-OH-MeIQx between primary cultures of myocytes and non-myocytes. Cells were exposed to the N-OH-MeIQx for an interval, then removed, and cell viability and lactate dehydrogenase (LDH) leakage were subsequently determined. LDH release increased as a function of N-OH-MeIQx dose for all cells. The results, however, showed that myocytes were significantly more sensitive than non-myocytes, such as fibroblasts, to the toxic effects of N-OH-MeIQx. We hypothesize that damage formed by these dietary components accumulate in working genes due to inadequate repair, thereby affecting heart cell viability, and we are currently examining the proficiency of different cell types to remove N-OH-MeIQx-DNA adducts. These results are therefore related to fundamental mechanisms regarding the effects of dietary factors on the regulation of cardiac myocyte homeostasis. Supported by the American Heart Association, Alabama Affiliate, No. G-930006.

A5-103 ACTIVATION OF THE MAMMALIAN STRESS-RESPONSE GENE *GADD153* BY OXIDATIVE STRESS, Kate Z. Guyton and Nikki J. Holbrook, Unit on Gene Expression and Aging, National Institute on Aging, Baltimore, MD 21224

The human *GADD153* gene, inducible by growth arrest and DNA damage, may function to control growth in response to stress signals. In the present study, the induction of *GADD153* by oxidative stress was investigated. H_2O_2 was shown to induce a time- and dose-dependent induction of *GADD153* mRNA in HeLa and WI38 cells. Further, H_2O_2 enhanced the expression of *GADD153* promoter-CAT reporter gene construct in stably transfected HeLa cells, suggesting a transcriptional mechanism for gene activation. The *GADD153* promoter-CAT reporter gene construct was also activated by a variety of free radical-generating compounds as well as thiol-reactive agents. The sulfhydryl agent sodium arsenite was particularly effective as an inducer of both *GADD153* mRNA as well as CAT activity in stable *GADD153*-CAT HeLa transfectants. The potency of sodium arsenite and other heavy metals as inducers of *GADD153* corresponded with their relative reactivity towards glutathione (GSH). The free radical scavenger N-acetyl cysteine blocked induction of *GADD153* mRNA by sodium arsenite as well as by H_2O_2, whereas GSH depletion by buthionine sulfoximine (BSO) pretreatment potentiated *GADD153* expression. Moreover, overexpresssion of GSH peroxidase in WI38 cells caused a diminution in the induction of *GADD153* by both H_2O_2 and sodium arsenite, indicating that the accumulation of intracellular hydroperoxides functions in gene activation by both of these compounds. Finally, the induction of *GADD153* by UV (254 nm) was partially blocked by N-acetyl-cysteine and weakly potentiated by BSO, suggesting that oxidative stress is a component of the cellular response to UV damage. Together these results suggest that oxidizing agents can transcriptionally activate *GADD153*, thus providing evidence that oxidant injury plays a key role in this molecular response to cellular stress.

A5-104 THE CHEMOPROTECTIVE EFFECTS OF CAFESTOL AND KAHWEOL: EFFECTS ON XENOBIOTIC METABOLISING ENZYMES, Anthony C. Huggett and Benoit Schilter, Department of Quality and Safety Assurance, Nestec Ltd Research Centre, Vers-Chez-les-Blanc, Lausanne, Switzerland.

The coffee-specific diterpenes cafestol and kahweol (C+K), which comprise up to about 10-15% of the lipid fraction of roasted coffee beans, have recently been shown to possess several important biological activities. For example they modulate serum cholesterol levels and they have been reported to have anticarcinogenic properties. The latter activity may be related to their ability to induce glutathione S-transferase (GST). However, little is known concerning their effects on other xenobiotic metabolising and detoxifying systems. In a sub-chronic feeding study in rodents we examined the influence of a mixture of C+K on the expression of various enzymes involved in xenobiotic metabolism. Analysis focused on liver samples from rats fed a mixture of C+K incorporated at four dose levels in the diet for 28 or 91 days. The expression of mRNAs specific for cytochromes P450s CYP1A1, CYP1A2, CYP2B1, CYP2B2 and CYP3A1 as well as for GST alpha sub-units Ya1, Ya2, Yc and the GST mu sub-units Yb1 and Yb2 were assayed. For this purpose highly selective synthetic oligomers capable of discriminating between structurally related gene products were employed. C+K induced a dose-dependent increase in the expression of mRNAs encoding the P450s CYP2B1, CYP2B2 and CYP3A1. The effects of C+K on the expression of liver GST was subunit-dependent. Compared to control animals, the content of mRNAs encoding GST subunit Ya1 was unaffected, Yb2 and Yc were increased slightly and Yb1 was markedly increased, consistent with an overall induction of the GST mu form. These effects were associated by a dose-dependent increase in total GST activity determined at the enzymatic level. As part of a validation of molecular markers we are investigating whether analogous effects are observed using human and rodent liver cell cultures.

A5-105 **ESSENTIAL ROLE OF POLY(ADP-RIBOSYL) ATION IN CELLULAR RECOVERY FROM OXIDATIVE STRESS**, Guy G. Poirier, Danièle Poirier, Serge Desnoyers, Sylvie Saint-Martin, Jean-Christophe Hoflack, and Girish M. Shah, Poly(ADP-ribose) Metabolism Group, Unit of Health and Environment, CHUL Research Center, 2705, Boulevard Laurier, Sainte Foy, Québec, Canada, G1V 4G2.

DNA base excision repair *in vitro* is dependent on the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which catalyzes synthesis of a polymer of ADP-ribose (pADPr) from substrate NAD (1,2). However, direct proof for essential role of PARP in DNA repair was unavailable perhaps due to use of inefficient inhibitor of PARP. We report that 1,5-dihydroxy-isoquinoline (DHQ) is a very potent inhibitor of PARP in intact cells. We measured a 100-200 fold stimulation of pADPr synthesis in C3H10T1/2 cells responding to active oxygen by a very sensitive technology. DHQ (0.1 mM) inhibited the oxidant-stimulated pADPr synthesis down to 1.7 times the control (untreated cells), whereas there was no reduction in clonogenicity of the cells. It was only at 1 mM of DHQ, when we observed no pADPr synthesis (0.6 times the control), that there was a dramatic inhibition (98%) of clonogenicity of oxidant-injured cells. In contrast, the incomplete PARP inhibitor benzamide was unable to potentiate the cytotoxicity of oxidative stress in the same cell line (3). This is a confirmation of earlier observation (2) that even minor automodification of PARP, will prevent it from reattaching to DNA strand breaks and thus permit DNA repair. Only a verifiably complete inhibition of PARP activity by DHQ allowed us to demonstrate the essential role of PARP in DNA repair and cellular recovery after an oxidative stress.

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A5-106 INVOLVEMENT OF THE KU AUTOANTIGEN IN THE CELLULAR RESPONSE TO DNA DOUBLE-STRAND BREAKS, W. Kimryn Rathmell, Vaughn Smider, Michael Lieber and Gilbert Chu, Department of Medicine, Stanford University Medical Center, Stanford, CA 94305

The Ku autoantigen is a DNA end-binding heterodimer of 70 and 86 kDa. However, despite extensive characterization the function of Ku *in vivo* has remained elusive. An electrophoretic mobility shift assay and Ku antisera were used to show that Ku or a closely related protein was deficient in three mutant hamster cell lines from X-ray sensitive complementation group 5, one of three complementation groups characterized by defects in DNA double-strand break repair and V(D)J recombination [W. K. Rathmell and G. Chu, Mol. Cell. Biol. 14, 4741 (1994), W. K. Rathmell and G. Chu, Proc. Natl. Acad. Sci. USA 91, 7623 (1994)]. Furthermore, transfection of cDNA encoding the 86 kDa but not the 70 kDa subunit of Ku restored DNA end-binding activity, X-ray resistance, and V(D)J recombination activity to these mutant cells. In addition, the availability of mutant cells rescuable by the Ku protein makes possible further insight into the processes of DNA double strand-break repair and V(D)J recombination.

These results establish a role for Ku in DNA repair and recombination. As the DNA binding subunit of a DNA-dependent protien kinase, Ku may also couple DNA damage to a signaling pathway, perhaps for cell cycle arrest or apoptosis.

A5-107 THE LOW LEVELS OF SOME TRANSCRIPTION FACTORS BINDING IN NICKEL RESISTANT CELLS CORRELATES WITH SILENCING OF THROMBOSPONDIN EXPRESSION, Konstantin Salnikow, Min Gao, Sheng Wang and Max Costa, Department of Environmental Medicine and The Kaplan Comprehensive Cancer Center, New York University Medical Center, New York, NY 10016

In attempt to understand the molecular mechanism of nickel carcinogenesis, we have used subtractive hybridization to clone five genes which were inactivated in nickel transformed cells. Two of these sequences, thrombospondin and peroxidase, were also found to be inactive in nickel resistant cells. Since thrombospondin is antiangiogenic, its inactivation is an important contribution toward tumor formation **in vivo**. Thrombospondin silencing was not due to methylation of its promoter region based upon analysis with methylation sensitive restriction endonucleases (HpaII and HhaI) and sodium bisulfite treatment. Analysis of the thrombospondin promoter revealed that it contained a number of known regulatory sequences including AP-1, ATF, NF-kB, Sp1 responsive elements. Using mobility shift, we have analyzed binding activity of nuclear extracts from cell lines expressing and not expressing thrombospondin. Very low binding activity of Sp1, AP-1, and NF-kB was found in cell lines that do not express thrombospondin. Other transcription factors, E2F and Oct1, did not show such differences in binding. We suggest that changes in composition and assortment of transcription factors take place in nickel transformed or resistant cells and such changes lead to inactivation of genes such as thrombospondin.

A5-108 ANALYSIS OF *MDR*1 GENE INDUCTION BY TRANSIENT EXPOSURE TO CYTARABINE, Alexander A. Shtil and Igor B. Roninson, Department of Genetics, University of Illinois at Chicago, Chicago, IL 60612-7309

Expression of the multidrug transporter, P-glycoprotein, encoded by the human *MDR*1 gene, can be induced by cellular damage with various cytotoxic agents, including those that are not transported by P-glycoprotein. *MDR*1 induction can be prevented by non-specific inhibitors of protein kinase C (PKC) (Chaudhary and Roninson, JNCI 4, 281-290, 1993). We have now analysed the process of *MDR*1 induction in human H9 T-cell leukemia line, which normally expresses *MDR*1 at exceedingly low levels. *MDR*1 induction in H9 cells was measured by RT-PCR after treatment with cytarabine (AraC), a cytotoxic agent resistance to which is not affected by P-glycoprotein. Exposure to 25 μM AraC resulted in gradual increase in the cellular levels of *MDR*1 mRNA, which became detectable 6 hours after the addition of the drug and reached plateau at about 48 hours. At a higher dose of AraC (75 μM), *MDR*1 induction was apparent after incubation for as little as 1 hour. The increase in *MDR*1 expression was prevented by the addition of actinomycin D at different time points, suggesting that *MDR*1 induction was most likely regulated at the level of transcription. AraC-induced *MDR*1 expression decreased 48 hours after removal of the drug but remained higher than in untreated cells for a prolonged period of time (at least 6 weeks). We have also tested the effects of different inhibitors of serine/threonine or tyrosine protein kinases on *MDR*1 induction by AraC. The effect of AraC was partially or completely prevented by serine/threonine kinase inhibitors effective against PKC and by inhibitors of those tyrosine kinases that are known to promote PKC activation. Our results are consistent with a model that stress induction of the multidrug transporter occurs at the level of transcription and is mediated by PKC. Supported by NCI Oncology Research Faculty Development Program (AAS) and grant CA40333 (IBR).

A5-109 Transcriptional Regulation of the Rat *mdr1b* Gene by Carcinogens Jeffrey A. Silverman and Barbara A. Hill
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The expression of rat *mdr1b* gene is increased in primary rat hepatocytes and the H4-IIE hepatoma cell line by exposure to carcinogens such as aflatoxin B_1, N-acetoxy-2-acetylaminofluorene and methyl methanesulfonate. Nuclear run-on experiments indicated that the higher steady state levels of *mdr1b* mRNA are due to an increase in transcription. The 5' flanking region of the *mdr1b* gene was isolated, sequenced and functionally characterized in transient and stable transfection assays. A single transcription start site was identified for this gene; no alternate start sites were used following induction with aflatoxin B_1. Deletion analysis of this promoter demonstrated that the sequence between -214 to -178 is critical for basal promoter activity. This region does not contain any consensus binding sites for previously identified transcription factors. A negative regulatory region was also identified between -940 to -250. No specific carcinogen-responsive element was identified; the xenobiotic response required a broad region of the promoter. These data suggest that the carcinogen induction of *mdr1b* expression is mediated through sequences overlapping with or identical to the basal promoter element.

A5-110 GENOTOXICOLOGY OF BENZO[A]PYRENE-7,8-DIHYDRODIOL AND BENZO[A]-PYRENE-7,8-DIHYDRODIOL-9,10-EPOXIDE IN GENETICALLY ENGINEERED HUMAN CELLS, J. Christopher States[1], TaiHao Quan[1], John J. Reiners, Jr[2] and Sandra J. Culp[3], [1]Center for Molecular Medicine and Genetics and [2]Institute of Chemical Toxicology, Wayne State University, Detroit, MI 48201 and [3]Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079

We genetically engineered human fibroblasts to express human CYP1A1 [6] to address whether treatment of cells with an ultimate mutagen (i.e. BPDE) is an appropriate model for a promutagen (i. e. BPD) that requires metabolic activation. The genotoxicological effects of BPDE intracellularly produced by CYP1A1 metabolism of BPD and extracellularly supplied BPDE were compared. Both BPD and BPDE were preferentially cytotoxic to XPA cells relative to normal cells. BPD caused a larger differential cytotoxicity between XPA and normal cells than BPDE. At comparable cytotoxicity in both XPA and normal cells, BPD induced more mutants and more DNA adducts than BPDE. DNA adducts were identified and quantified by ^{32}P-post-labelling. 10-β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo-[a]pyrene was the principal adduct formed by BPD and BPDE. At similar levels of DNA adducts in XPA cells, BPD and BPDE-induced cytotoxicity were similar, but BPD induced a 3-fold higher HPRT mutation frequency. At similar levels of adducts in normal cells, BPD induced lower cytotoxicity and mutation frequency. The results suggest that CYP1A1-metabolized-BPD may form adducts preferentially in transcriptionally active genes and/or that the intracellular concentration of BPDE may influence the balance between cytotoxicity and mutagenicity.

A5-111 DOSE DEPENDENT DIFFERENTIAL REPAIR OF (+)-*ANTI*-BPDE-DNA ADDUCTS IN EUKARYOTIC CELLS Sundaresan Venkatachalam, Mikhail Denissenko and Altaf A. Wani, Department of Radiology and Biochemistry Program, The Ohio State University, Columbus, OH 43210

(+)-7α,8ß-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(*a*)pyrene [(+)-*anti*-BPDE], one of the various metabolites of the ubiquitous carcinogen, benzo(*a*)pyrene, has been implicated in the process of chemical carcinogenesis via the formation of (+)-*anti*-BPDE-DNA adducts. Polyclonal antibodies specific for (+)-*anti*-BPDE-DNA adducts were developed and utilized to analyze the interaction of this electrophilic genotoxin with DNA *in vitro* in different media conditions and follow adduct repair in eukaryotic cells. Non-competitive enzyme linked immunosorbent (ELISA) and immunoslotblot (ISB) assays were performed for the immunoanalyses. ISB assays detected adducts induced by doses of < 1 nM *anti*-BPDE corresponding to 2 adducts/10^7 nucleotides. Rapid hydrolysis of (+)-*anti*-BPDE (~ 30 minutes) was observed in Hank's BSS and DMEM resulting in < 5% of the initial adduct induction compared to that of (+)-*anti*-BPDE in 95% ethanolic medium. Repair proficient human fibroblasts showed dose dependent rates of adduct removal. At low and intermediate levels of initial damage (4 and 20 adducts/10^6 nucleotides), 50$\pm$2 and 42$\pm$1.2 % repair was observed in 8 hours, resp.. With higher doses corresponding to initial adduct/nucleotide ratio of 100$\pm$3 and 160$\pm$1.9/10^6, only 35 and 10$\pm$3.7% of the adducts were repaired in 24 hours, respectively. Repair deficient XP fibroblasts (group A), while repairing ~ 10 % of the adducts at very low doses (0.1 μM corresponding to 4$\pm$0.26 adducts/10^6 bases), did not exhibit any loss of antibody binding sites in DNA at higher doses after 24 hours following exposure. These highly sensitive and mono-adduct specific antibodies allow the study of processing and quantitation of very low levels of (+)-*anti*-BPDE-DNA adducts in various biological systems.

A5-112 EFFECTS OF 2,3,7,8-TCDD ON GENE INDUCTION VIA THE RETINOIC ACID SIGNALING PATHWAY, Wayde M. Weston, Paul Nugent, and Robert M. Greene, Department of Anatomy, Pathology, and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107

The polychlorinated aromatic hydrocarbon 2,3,7,8-tetrachlorodibenzo-p-dioxin, commonly referred to as dioxin or TCDD, has been shown to cause cleft palate in mice. TCDD displays an interesting interaction with another cleft palate teratogen, retinoic acid (RA): palatal clefts can be observed in 100% of offspring of mothers given combined doses of TCDD and RA at levels far below those required for either agent to produce clefting if given singly. This synergy strongly suggests that the signaling pathways controlled by these agents converge at one or more points in cells of the developing craniofacial region. In this study, we examined the effects of TCDD on induction of specific gene expression via the RA signaling pathway in murine embryonic palate mesenchyme (MEPM) cells. MEPM cells were treated with RA (1.65 μM) and TCDD (10 nM), singly or in tandem, for 24 hours and total RNA extracted from the cells. Northern blot analysis was carried out for the cellular retinoic acid binding proteins I and II (CRABP-I and II) and the nuclear retinoic acid receptor β (RARβ). CRABP-II and RARβ were strongly induced in MEPM cells by RA alone. TCDD alone had no effect on basal levels of expression of either gene. Combined treatment with TCDD and RA showed little or no increase over control levels of CRABP-II or RARβ expression, demonstrating that TCDD is able to interfere with the induction of genes specifically induced by RA. TCDD's effects on RA-mediated gene expression appeared to be limited to inhibition of induction, as down-regulation of CRABP-I by RA was unaffected by TCDD. These results show that 1) TCDD is able to influence the ability of RA to induce gene expression, and 2) TCDD may indirectly affect more downstream endpoints of RA action mediated by CRABP-II and RARβ. Supported by PHS grants DE05550 and DE08199 to RMG and DE11176 to WMW. PN is supported by NRSA DE05633.

A5-113 DAMAGE SPECIFICITIES OF THE MAJOR HUMAN APURINIC ENDONUCLEASE (APE), D.M. Wilson III, D.M. Wilson, M. Takeshita*, A.P. Grollman*, B. Demple, Dept. of Molecular and Cellular Toxicology, Harvard School of Public Health, Boston, MA 02130/*Dept. of Pharmacological Sciences, SUNY-Stony Brook, New York 11794

AP sites and single-strand DNA breaks with 3'-termini blocked by nucleotide fragments are two prominent forms of damage that result from attack of DNA by reactive oxygen species. These two potentially mutagenic lesions are repaired by class II AP endonucleases. The major human AP endonuclease (Ape) has been identified and its structural gene isolated.

A physiological role for Ape in repairing alkylation-induced AP sites is demonstrated by complementation experiments performed in repair-deficient (*Δapn1*) yeast. We are currently investigating whether Ape can also suppress the mutator phenotype of *Δapn1* yeast strains.

Experiments with synthetic oligonucleotide substrates have provided insight into the mechanism of damage recognition and incision by Ape. The specificity (k_{cat}/K_m) of Ape for duplex DNAs containing synthetic AP site analogs [tetrahydrofuranyl (F), propanyl (P), and ethanyl (E)] is F > P > E. The base in the opposite strand appears to play little role in influencing the rate of DNA cleavage. In contrast, the nucleotide context both 5' and 3' to these adducts as well as the position of the lesion relative to the ends of the DNA duplex have significant effects. These data will be discussed in the context of a model for DNA damage recognition by a critical repair enzyme. (This research is supported by an NCI post-doctoral fellowship to DMWIII)

A5-114 *IN VIVO* INDUCERS OF OXIDATIVE STRESS ACTIVATE soi28 AND zwf PROMOTERS *IN VITRO*, **Ralph Yamamoto, Catherine L. Zandonella and Dale E. Johnson, Department of Toxicology, CHIRON Corp. Emeryville, CA 94608.**

Xenobiotics that generate free radical moieties and/or superoxide *in vivo* induce toxicities associated with lipid peroxidative mechanisms. This current study was designed to detect oxidative stress-responsive genes *in vitro* for future use in screening combinatorial libraries of novel compounds. The oxidative stress generating properties of paraquat, 4-nitroquinoline oxide (4-NQO), and 1-nitronaphthalene (1-NN) were investigated using the bacterial gene expression (Pro-Tox, Xenometrics) and Yeast mutagenic assays (Yeast-Del, Xenometrics). Paraquat up-regulated soi28 and zwf gene expression in a dose-dependent fashion and the antioxidant, (+)-alpha tocopherol (vitamin E), suppressed peak induction levels. In dose response experiments using soi28 and zwf gene reporters, high concentrations of 4-NQO consistently resulted in "bell-shaped" induction curves. Results with 4-NQO and vitamin E suggest that increasing concentrations of 4-NQO in the assay may reduce the response at high concentrations through autoquenching. The requirement for metabolic activation to toxic and mutagenic moieties was also examined. Incubation of 1-NN with S-9 from aroclor-induced rat liver microsomes resulted in 3-4 fold increase in soi28 and ada gene expression as well as a 4-fold increase in yeast recombination. All of these *in vitro* findings, including identification of oxidative stress genes, protection by vitamin E, and the requirement for metabolic activation are consistent with known *in vivo* mechanisms of toxicity for these xenobiotics.

Receptor Mediated Toxicity; Apoptosis

A5-200 ORIENTATION OF THE AHR/ARNT HETERODIMER ON THE XENOBIOTIC RESPONSE ELEMENT OF *CYP1A1* BY UV CROSSLINKING ANALYSIS

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The DNA binding form of the aryl hydrocarbon receptor (AHR) complex is a heterodimeric transcription factor composed of the 90 kDa AHR monomer and the 87 kDa Ah receptor nuclear translocator (ARNT) protein, both of which belong to the family of bHLH transcription factors. The AHR complex binds a variety of environmentally important carcinogens such as chlorinated polycyclic aromatic hydrocarbons (eg. dioxin). This ligand bound form of the receptor activates several genes involved in xenobiotic metabolism by direct contact with transcription enhancer elements called Xenobiotic Responsive Elements (XREs) found within the promoter regions of these genes. Other bHLH family proteins bind an E-box consensus sequence (5'-CANNTG-3'), which resembles the XRE only in the 3' end. We have previously demonstrated that both AHR and ARNT directly contact XRE 1 of the *CYP1A1* gene. We used UV crosslinking in conjunction with a panel of dBrU substituted XREs to define which base pairs in the XRE core element (5'-TTGCGTG-3') are in close proximity to each subunit of the AHR complex. The ARNT protein bound to the 3' end of the XRE core where half of an E-box sequence (-GTG-3') is present. The AHR binds to the second position from the 5' end of the XRE core. Our results are consistent with the fact that sequence similarities occur between the basic regions of ARNT and "class B" E-box binding proteins, such as USF and Max, whereas the basic region of AHR is considerably different.

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A5-201 LEAD EXPOSURE DURING PREGNANCY AND LACTATION MODIFIES EFFECTS OF DIETARY CALCIUM ON KIDNEY CALBINDIN D28K, John Bogden, Francis Kemp, Shenggao Han, and Sylvia Christakos, Departments of Preventive Medicine and Biochemistry and Molecular Biology, UMDNJ-New Jersey Medical School, Newark, NJ 07103

Interaction with proteins is the fundamental mechanism by which lead produces toxicity. Since pregnancy and lactation have profound effects on calcium metabolism, we studied the potential for lead exposure during pregnancy and lactation to modify the effects of dietary calcium on levels of the calcium binding protein calbindin D28K in the kidney. This vitamin D-dependent protein binds four lead atoms per molecule with greater affinity than its binding of calcium; its synthesis is enhanced by diets low in calcium. Pregnant SD rats (n=43) were randomly assigned to one of 6 treatment groups of 7-8 rats each. Half were fed diets of low (0.1%), normal (0.5%), or high (2.5%) Ca and exposed to 250 mg/L of lead in their drinking water for the duration of pregnancy (21 $\pm$ 2 days) and the first week of lactation. Control groups were fed the same diets without lead exposure. Blood and organs were obtained from day old pups, week old pups, and the dams. There were statistically significant effects of lead exposure (ANOVA, $p < 0.001$) and dietary calcium ($p < 0.001$) on blood and kidney lead concentrations and renal calbindin D28K levels, as well as significant lead/calcium interactions. Lead concentrations were highest in pups and dams fed the low calcium diet and lowest when dams were fed the high calcium diet. Calbindin D28K concentrations were significantly reduced by lead exposure for dams fed low and normal, but not high, dietary calcium. The results demonstrate that responses of calbindin D28K to dietary calcium are modified by lead exposure during pregnancy and lactation. (Supported in part by Reproductive Hazards in the Workplace, Home, Community and Environment Research Grant No. 15-FY93-0659 from the March of Dimes Birth Defects Foundation.)

A5-202 **PROTECTION AGAINST TOXIN INDUCED APOPTOSIS BY GROWTH FACTORS.**

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It has been suggested that growth factors can inhibit apoptosis, a form of programmed cell death by providing a separate and distinguishable signal for survival (1). As accumulating evidence suggests that disparate toxins can induce apoptosis (2) we tested the hypothesis that growth factors could protect against toxin induced apoptosis. Experiments showed that exposure of Rat-1 fibroblasts to 130mM dimethylformamide, a solvent of wide occupational exposure, induced cell death characteristic of apoptosis. Concomitant addition of IGF-1 protected cells against DMF induced toxicity, despite being non-mitogenic in this system. Conversely, EGF (a mitogen for Rat-1 fibroblasts) did not protect against toxin induced cell death. These results suggest that it may be possible to shift the toxicity threshold of a chemical by providing cells with receptor mediated signals which promote survival.

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A5-203 ANALYSIS OF AH RECEPTOR FUNCTIONAL DOMAINS INVOLVED IN AH RECEPTOR COMPLEX MEDIATED SIGNAL TRANSDUCTION

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The aryl hydrocarbon receptor (AhR) binds a variety of environmentally important carcinogens such as chlorinated polycyclic aromatic hydrocarbons (eg. TCDD) and polychlorinated biphenyls, and activates transcription of several genes involved in xenobiotic metabolism, including *CYP1A1* and *CYP1A2*, UDP-glucuronosyl-transferase and glutathione-S-transferase. After subcellular fractionation, the unliganded receptor complex is found in the cytosol. This complex contains the Ah receptor (AhR), the 90 kDa heat shock protein (HSP90) and perhaps other, as yet unidentified, proteins. Upon ligand binding, a dimer of AhR and the Ah receptor nuclear translocator (ARNT) protein is found in the nucleus, where it binds to Xenobiotic Responsive Elements (XRE) which control transcription of the *CYP1A1* gene. AhR and ARNT both belong to the bHLH family of transcription factors and also contain the "PAS"-region, a second putative dimerization domain also found in two Drosophila proteins, PER and SIM, which are involved in the regulation of circadian rhythm and early CNS development, respectively.

We have identified functional domains of the AhR for ligand binding, dimerization with ARNT, XRE binding, and *in vivo* functionality, by means of site-directed mutagenesis.

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A5-204 EFFECTS OF ALLOGENIC SERUM ON THE IN VITRO IMMUNE RESPONSE : Ah RECEPTOR STRUCTURE-IMMUNOTOXICITY RELATIONSHIPS, N. Harper, M. Steinberg, and S. Safe, Dept. of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843-4466

Previous studies from this laboratory have demonstrated a lack of Ah receptor structure-immunotoxicity relationships in splenocytes cultured with fetal bovine serum; these results were not consistent with the observed in vivo immune response. In this study, the in vivo and in vitro immunsuppressive effects of Ah receptor agonists were compared using B6C3F1 female mice and mouse splenocytes cultured with allogenic mouse serum. The Mishell-Dutton model for in vitro immunization to trinitrophenyl-lipopolysaccharide (TNP-LPS) was used. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), 1,2,3,7,8-PeCDF, 1,3,6,8-TCDF, 3,3',4,4',5-pentachlorobiphenyl (pentaCB), or 3,3',4,4',5,5'-hexaCB resulted in a dose-dependent suppression of the splenic plaque forming cell (PFC) response both in vivo and in vitro. The effective dose required to decrease 50% (ED-50) of the immune response to 2,3,7,8-TCDD, 2,3,4,7,8-peCDF (PeCDF), 1,2,3,7,8-PeCDF, 1,3,6,8-TCDF, 3,3',4,4',5-pentachloro-biphenyl (PentaCB), or 3,3',4,4',5,5'-HexaCB was 4.8, 4.7, 1695, 348,000, 21, and 19 nmol/kg in vivo respectively, and was 7.7, 9.0, 122, 1729, 16, and 13 nM in vitro respectively. The structure-immunotoxicity relationships for the congeners tested were consistent with their relative binding affinities for the Ah receptor and there was an excellent correlation between the in vitro and in vivo immune responses ($r > 0.99$). These results show that splenocyte culture with allogenic mouse serum is necessary for mimicing the in vivo immune response and that this in vitro assay can detect immune modulation by Ah receptor agonists.

A5-205 LOCALIZATION AND SUBUNIT STRUCTURE OF THE Ah RECEPTOR IN HEPA 1c1c7 CELLS, Gary H.Perdew, Huey-Shang Chen, Norman G.Hord, and Sheo S. Singh, Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907.

The aryl hydrocarbon receptor (AhR) is a unique ligand-activated bHLH- transcription factor which, in association with the bHLH protein called the aryl hydrocarbon receptor nuclear translocator (Arnt), regulates the transcription of specific genes in a tissue- and species-specific manner. Monoclonal antibodies against the AhR were used for immunocytochemical analysis in mouse hepatoma cell line Hepa 1c1c7 (Hepa 1) cells. Laser scanning confocal microscopy revealed the AhR to be localized in both the cytoplasm and nucleus of Hepa 1 cells. Dioxin treatment of Hepa 1 cells caused a time-dependent increase in the amount of AhR associated with the nucleus. The presence of AhR was detected using anti-AhR MAb RPT1.Sucrose density gradient analysis of cytosolic and nuclear extracts from TCDD-treated and control Hepa 1 cells indicated the presence of the 9S form of the AhR complex in the cytosol and both 6S and 9S forms of the AhR in the nucleus.The 9S AhR in [^{35}S]-methionine labeled Hepa 1 cellular extracts was immunoprecipitated with anti-AhR polyclonal antibodies. Three radiolabeled bands were detected on western blots 97, 86/84, and 43 kDa. Antibodies were used to identify the AhR and hsp90 bands. Cross-linking studes revealed that the AhR is composed of four subunits. These data support the following conclusions: 1) the AhR is present in both the nucleus and the cytoplasm of Hepa 1 cells, 2) both the 6S and 9S forms of the AhR exists in the nucleus of cells after TCDD treatment, and 3) the 9 S cytosolic form of the AhR is a tetramer composed of the ligand binding subunit, a dimer of hsp90, and a 43 kDa protein.

A5-206 DRUG-INDUCED INTERFERENCE OF NEURO-RECEPTOR-MEDIATED CELLULAR SIGNALLING PROCESSES AS AN INDEX OF NEUROTOXICITY, Achyut M. Sinha, Vasanthi Ramachandran, Philomena and Bertha Elias, Astra Research Centre India, P.O.Box 359, Malleswaram, Bangalore 560 003, India.

Recent advances in the characterization of receptor subtypes and their implication in the etiology of certain brain disorders may enable us to develop direct, rapid and highly sensitive in vitro assays for testing drugs for brain toxicity. The traditional use of animal models are often unreliable, particularly in addressing end-points of brain toxicity caused by low doses of test compounds. To this end, we have constructed several mammalian recombinant cell lines stably expressing Dopamine D2 receptor subtypes. These cell lines have been studied in detail with respect to their agonist/antagonist specificity and effector-coupling pathways. Subtype-specific Dopamine D2 receptor malfunctioning has been implicated in the cause of certain specific brain disorders such as Parkinson's disease, schizophrenia and tardive dyskinesia. To date, no drug is available that is exclusively D2 receptor subtype specific, and as such is not highly selective for the treatment. Using the recombinant D2 receptor cell lines as well as other neural cell lines of known characteristics, we have initiated model studies to test the effect of known dopaminergic neuroleptics and neurotoxins on cellular signalling events mediated by the individual receptor subtypes. We propose that our approach will help to a great extent in evaluating these neuroleptics for subtype specificity, and, in principle, can be extended to test candidate drugs for brain toxicity.

A5-207 THE RAT CYTOCHROME P450IVA1 GENE IS REGULATED BY THE PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-ALPHA. AT THE TRANSCRIPTIONAL LEVEL. Jonathan D. Tugwood, Thomas C. Aldridge and Stephen Green, Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, U.K.

The peroxisome proliferators are a diverse class of rodent hepatocarcinogens that include industrially important compounds such as hypolipidaemic drugs and plasticisers. Since the original isolation of a peroxisome proliferator activated receptor (PPAR) from mouse liver (Issemann & Green Nature 347:645-650, 1990), significant advances have been made in our understanding of how these compounds may act at the molecular level. In particular, it has been established that PPARs regulate at the transcriptional level several peroxisomal enzymes involved in the β-oxidation of fatty acids. This transcriptional regulation is achieved via the formation of heterodimers between PPARs and the retinoid X receptors, which recognise specific DNA elements in the promoters of the regulated genes.

Of the enzymes upregulated *in vivo* in rats in response to peroxisome proliferators, the cytochrome P450IVA1 is induced the most rapidly, suggesting the possibility that this enzyme is somehow involved in the direct activation of PPARs and is not iself regulated by these receptors. We have shown that the rat cytochrome P450IVA1 is indeed transcriptionally regulated by PPARα, and that this is achieved through the formation of heterodimers with RXR. Furthermore, the DNA element mediating this activation shows structural conservation with that mediating the transcriptional activation of the rabbit cognate CYP4A6 gene (Muerhoff *et al.*, J. Biol. Chem. 267:19051-19053, 1992), in that two PPRE-like elements are present. This suggests that the *in vivo* regulation of this enzyme may involve factors other that PPARs and RXR.

A5-208 IDENTIFICATION OF THREE NOVEL TCDD-RESPONSIVE GENES IN HEP G2 CELLS USING DIFFERENTIAL mRNA DISPLAY RT-PCR, Xiaohong Wang, Peter K.W. Harris and Richard L. Voorman, Drug Metabolism Research, The Upjohn Company, Kalamazoo, MI 49001

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a well-known environmental contaminant and induces broad toxicological and biological responses in humans and animals. The search for TCDD-responsive genes is important to understand the mechanism of TCDD-induced responses. Using a conventional subtraction library to identify these genes can be very tedious and inefficient. Differential mRNA display RT-PCR (DD RT-PCR) offers the promise of a very powerful tool to identify genes which are regulated or responsive to certain receptors or chemicals such as TCDD. Treatment of Hep G2 cells with TCDD resulted, as expected, in expression of CYP1A1 mRNA which was differentially displayed on a gel with a homologous primer. Further TCDD treatment followed by DD analysis of a gel with a group of different primers revealed significantly different pattern from the control for a number of mRNA's. Twenty four differentially displayed mRNA's were isolated and reamplified. The reamplified PCR products were directly sequenced to give immediate DNA sequence information. A Genebank search revealed that only one had been cloned previously; the remaining 23 are currently unknown. Northern blot analysis revealed that at least 3 clones showed repression by TCDD in a time-dependent manner. These results show that the application of DD RT-PCR to the study of TCDD-induced responses could be very useful in the discovery of as yet unknown genes important for TCDD toxicity. The further characterization of these genes will be discussed.

Molecular Mechanisms of Carcinogenesis; Transgenic Models of Toxicity

A5-300 GERM CELL MUTAGENESIS STUDIES WITH *λlacZ* TRANSGENIC MICE (MutaMouse) TREATED WITH N-ETHYL-N-NITROSOUREA. Robert A. Baan and Joost H.M. van Delft, Department of Genetic Toxicology, TNO Nutrition and Food Research Institute, P.O.Box 5815, 2280 HV Rijswijk, The Netherlands

For several years transgenic mice have been available for mutagenesis studies in every organ, both in somatic cells and in germ cells. In *λlacZ* mice (MutaMouse) the target gene for mutagenesis is *lacZ*, of which multiple copies are present per nucleus, integrated in the genome. Mutants are scored with a positive selection method after transfer of the *λlacZ* vector into *E. coli*. Comparison of data obtained for the transgenes in these mice with those of endogenous genes is of interest for validation of this animal model. One of the classical methods to study germ cell mutagenesis is the specific locus assay (SLA). In this assay the offspring of mice at various time points after genotoxic treatment of the males are screened for the occurrence of mutations. We investigated germ cell mutagenesis in male *λlacZ* mice induced by N-ethyl-N-nitrosourea (ENU) and compared our data with those published for the SLA. In order to achieve an optimal comparison, mutagenesis was studied in mature spermatozoa isolated from *epididymis* and *vas deferens*.

For animals treated with ENU (150 mg/kg; i.p.) the mutant frequency (MF) at 0.1, 7, 50 and 100 days post-treatment is 33, 28, 124 and 370×10^{-6}, whereas for control animals the MF is 41×10^{-6} (0.1-100 days). This means that only an increase of the MF occurs in stem cells (9-fold) and not in post-stem cells, in contrast to the results of the SLA which showed an induction of mutations during both phases of spermatogenesis (48- and 6-fold). The larger induction in the SLA may be explained by a lower background MF, namely $8\text{-}12 \times 10^{-6}$ *vs* 41×10^{-6} for the transgenic mouse assay. The limited increase of MF at 50 days post-treatment compared to 100 days, is probably due to a delay of spermatogenesis (normally around 42 days) caused by a cytotoxic effect of ENU on the testis. This was confirmed by our observation that O^6-ethylguanine levels in spermatozoan DNA remained approximately the same between 0.1 and 50 days and was considerably lower at 100 days.

The MF at 100 days post-treatment for ENU at 0, 25, 50 and 150 mg/kg is 57, 79, 91 and 370×10^{-6}. This indicates a threshold for mutation induction at low dosages and is in accordance with the SLA data.

In summary, our data suggest that *λlacZ* transgenic mice can be used to study germ cell mutagenesis in stem cells but not in post-stem cells, and that this assay is less sensitive than the SLA but yields comparable stem cell data.

A5-301 INACTIVATION OF RETINOBLASTOMA PROTEIN ASSOCIATED WITH NICKEL-INDUCED TRANSFORMATION OF HUMAN CELLS, W. Karol Dowjat, X. Lin and M. Costa, Department of Environmental Medicine and The Kaplan Comprehensive Cancer Center, New York University Medical Center, New York, NY 10016

Following treatment of the human osteoblastic HOS-85 cell line with crystalline NiS, several anchorage-independent clonal cell lines have been obtained. Acquisition of transformed phenotype was associated with changes in properties of retinoblastoma protein (pRb). Retinoblastoma protein in Ni-transformed cell lines was hypophosphorylated, located mostly in the cytoplasm and unable to form complexes with SV40 large T antigen. Also, Ni-transformed cells showed significantly lower expression of the Rb gene. Reintroduction of normal Rb gene resulted in marked reduction of cell growth, suppression of ability to grow in soft agar and appearance of cells with senescence-like morphology. RT-PCR sequence analysis of Rb gene in one Ni-transformed clone did not reveal the presence of any mutation. A novel pRb-associated protein present only in transformed cells has been identified. Its presence together with low level of pRb expression may explain why despite an apparent lack of mutation within the Rb gene, the protein was rendered functionally inactive in Ni-transformed cells.

A5-302 EXPERIMENTAL STUDIES OF THE TOXICITY AND CARCINOGENICITY OF NICKEL SULFATE, NICKEL SUBSULFIDE, AND NICKEL OXIDE.
June K. Dunnick and Michael R. Elwell, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

The relative toxicity and carcinogenicity of nickel sulfate hexahydrate ($NiSO_4.6H_2O$), nickel subsulfide (Ni_3S_2) and nickel oxide (NiO) was studied in F344/N rats and $B6C3F_1$ mice after inhalation exposure for 6 hr/day, 5 days/week for 13 weeks or 2 years. These chemicals were all toxic to the respiratory tract and the order of toxicity was nickel sulfate >> nickel subsulfide> nickel oxide. The water-soluble nickel compound is thought to be more toxic than the water-insoluble nickel compounds because it readily diffuses across the cell membrane and interacts with cytoplasmic proteins. Respiratory toxicity to these nickel compounds occurred below the current TLVs for water-soluble nickel compounds (0.1 mg Ni/m3) or water-insoluble nickel compounds (1 mg/m3). Preliminary findings from the evaluation of tumor response in the respiratory system indicated that the water-insoluble nickel compounds were more carcinogenic than the water-soluble nickel compounds. These findings agree with the results from other studies (Lee et al., Environ. Mol. Mut. 21:365-371,1993; Costa et al., Sci. Total Environ. 148:191-199,1994) which show that water-insoluble nickel compounds are phagocytized, and the vacuoles containing the nickel migrate to the nuclear membranes where they are dissolved, releasing nickel ions that proceed to effect DNA damage.

A5-303 TUMOR GROWTH AND THE ESTROGEN RECEPTOR IN A SERIES OF PITUITARY CLONAL CELL LINES IN RATS., Nariaki Fujimoto, Hiromitsu Watanabe and Akihiro Ito. Department of Cancer Research, Research Institute of Radiation Biology and Medicine, Hiroshima University. 1-2-3 Kasumi, Minamiku, Hiroshima, 734, JAPAN.

Four kinds of *in vitro* clonal pituitary tumor cell lines named MtT/Se, MtT/SM, MtT/S and MtT/E (established by Inoue *et al.* in 1990), each of which shows different sensitivity to estrogen on proliferation, were inoculated into fat pad of ovariectomized and estrogenized rats. They formed tumors with varied average latency ranging from 30 to 71 in ovariectomized rats and 13 to 63 days in estrogenized rats by a number of 10^6 cells' inoculation. MtT/Se was highly sensitive to estrogen for their growth, and MtT/SM also grew well in estrogenized rats. In MtT/S and MtT/E, there was no significant shortening of average tumor latency by estrogen. *In vivo*, cytosolic estrogen receptor (ER) levels of four types of clones, MtT/Se, SM, S and E were measured to be 452±66, 370±115, 260±16 and 83±8 fmol/mg protein, respectively. *In vitro*, however, the lowest ER level was noted in MtT/Se. Histologically, all of four tumors grown in rats were composed of homogeneous round cells, and MtT/Se contained particularly large nucleated cells. In MtT/E, it appeared to be changing into fibromatous cells. Three cell lines except MtT/E maintained the function of hormonal secretion *in vivo* as well as *in vitro*. Serum GH level was increased in rats with MtT/Se and MtT/S. Increased levels of both PRL and GH were measured in sera of rats with MtT/SM. Increases of hormones as well as tumor sizes were promoted by the estrogen.

A5-304 THE SYRIAN HAMSTER EMBRYO (SHE) CELL TRANSFORMATION SYSTEM – BOTH AN ACCURATE MULTISTAGE *IN VITRO* MODEL OF *IN VIVO* CARCINOGENESIS AND A HIGHLY PREDICTIVE CARCINOGEN SCREENING TOOL., Robert J. Isfort, David B.Cody, Gary A. Kerckaert, Claus-Jens Doersen and Robert A. LeBeouf, The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH 45239-8707

The Syrian hamster embryo (SHE) *in vitro* transformation system is a cellular transformation system which has been investigated both in terms of mechanisms of transformation and carcinogen prediction potential. Recently, we have modified the standard SHE assay so that it is easier to use, more predictive, and able to provide reagents for the study of intermediate transformation stages. In terms of carcinogen prediction, the SHE assay has a 80–90% concordance with carcinogenicity for a data set of over 250 carcinogens and noncarcinogens while overall, more than 500 chemicals have been evaluated for morphological transformation in the SHE assay. The strong concordance of transformation in the SHE assay with carcinogenicity has allowed us to use the SHE assay as an *in vitro* surrogate for *in vivo* carcinogenicity in order to identify the molecular mechanisms of transformation. Using SHE cell lines which represent the morphologically transformed, immortal, tumorigenic and tumor derived stages of transformation from multiple transformed lineages, we have identified multiple stage specific mutations which contribute to the SHE cell tumorigenic phenotype and which have direct counterparts in *in vivo* tumor models. These alterations include stage specific changes in: (1) autocrine growth factor production; (2) proliferative responses to mitogens and mitogenic inhibitors; (3) cellular differentiation state; (4) cellular cytoskeleton; (5) tumor suppressor genes; (6) oncogene function; and (7) 3D gel growth characteristics. Importantly, specific transformation stages have specific mutational profiles with all stage specific mutations being independent of the transformation mechanism. These results demonstrate that the SHE transformation system is an accurate *in vitro* model of *in vivo* carcinogenicity and as such is able to accurately predict, via cellular transformation, the carcinogenic potential of chemicals.

A5-305 RADIATION-INDUCED MICRONUCLEI IN p53-DEFICIENT AND NORMAL MICE. David Jacobson-Kram, Hans Raabe, Betsy Schadly, and D. L. Putman. Genetic and Cellular Toxicology Division, Microbiological Associates, Rockville, MD 20850.

Mutations in the p53 tumor suppressor gene are the most frequent genetic alteration observed in human tumors. One proposed function for the p53 protein is to monitor DNA for the presence of damage and prevent a cell from performing scheduled DNA synthesis on a damaged template. We have investigated this hypothesis by subjecting homozygous p53 deficient mice (TSG-p53®) and wild type controls to various doses of ionizing radiation and collecting bone marrow for quantitation of micronuclei at increasing times following exposure. Neither p53 deficient nor wild type animals showed increased frequencies of micronuclei when bone marrow was harvested at 6 hours after irradiation with 1, 2 and 3 Gy. Transgenic and wild type mice sacrificed 24 hours after irradiation showed increased frequencies of micronuclei with no concomitant alteration in the ratios of polychromatic erythrocytes (PCE) to total erythrocytes (TE) (a measure of toxicity). Wild type animals sacrificed 48 hours after irradiation showed no elevation in frequencies of micronuclei and severe reduction in the PCE:TE ratio. p53 deficient animals sacrificed at 48 hours showed dose-related increases in micronuclei up to 17.2 micronucleated PCEs/1000 cells at 3Gy with no change in the PCE:TE ratio. The results suggest: 1) Cells exposed to radiation after G_1 continue to cycle and produce micronuclei regardless of their p53 status. 2) p53 deficient cells irradiated before G_1 show increased chromosomal damage and no inhibition in the production of PCE while similarly treated wild type cells appear almost completely blocked. These observations support the hypothesis that p53 acts to detect DNA damage and arrest cells in G_1 to effect repair. Supported by NCI SBIR 1 R43 CA55471-0.

A5-306 *IN VIVO* INDUCTION OF HUMAN PHASE I AND PHASE II ENZYMES BY DIET. Morten A. Kall, Ole Vang, Ole Andersen and Jørgen Clausen Department for Life Sciences and Chemistry, Post Box 260, 4000 Roskilde, Denmark.

Previous studies have showed that the activity of phase I and phase II enzymes *in vivo* and *in vitro* may be affected by dietary compounds e.g. compounds derived from *Brassica* vegetables, onions, charcoal grilled meat etc. Previously, we have shown that the P450 1A2 activity in a group of 23 healthy nonsmoking men were significantly decreased (16 %) after a six days' intervention period with a diet low in known and suspected inducers[1]. As a continuation of this study we examined the induction effect of a diet rich in known inducers. The studies were designed to compare the effect of a customary home diet with : 1) a six days' intervention period with the low induction diet and 2) a 5-11 days' intervention period with a supplement of known dietary inducers to this diet. In the first study 33 volunteers had a charcoal grilled hamburger for lunch for five days after a six days' period with the low induction diet. P450 1A2 activity was determined prior to and after the period of ingestion of grilled meat. In the second study 18 volunteers received 500 gr. fresh broccoli every day for 11 days after a six days' period with the standard low induction diet. P450 1A2, 2E1 and GST μ were determined. The experiments were designed to evaluate interindividual variations in the genetic disposition to induction. This was done by comparing the degree of induction in groups of individuals receiving comparable amounts of dietary inducers. The P450 1A2 activity was expressed as the caffeine metabolic ratio, determined by HPLC analysis of urinary caffeine metabolites in a spot urine 6 hours after oral ingestion of 100 mg of caffeine. P450 2E1 activity was expressed as the ratio of 6-hydroxychlorzoxazone to chlorzoxazone assayed by HPLC analysis of plasma chlorzoxazone metabolites 2 and 4 hours after oral ingestion of 500 mg chlorzoxazone. Glutathion S-transferase μ was assayed in isolated lymphocytes with 1-chloro-2,4-dinitrophenol as substrate.

[1] M. A. Kall and J. Clausen: Dietary effects on mixed function P450 1A2 activity evaluated by caffeine metabolism in man. (Submitted)

A5-307 INDUCTION OF GENOMIC INSTABILITY IN NORMAL HUMAN BRONCHIAL EPITHELIAL (NHBE) CELLS BY α-PARTICLES, John F. Lechner, Christopher H. Kennedy, Robin E. Neft, and Noelle H. Fukushima, Inhalation Toxicology Research Institute, Albuquerque, NM 87185

Inhaled α-emitting radon daughters constitute a risk for development of lung cancer. In this project, cultured NHBE cells are being used as a model to determine if sustained genomic instability is an early feature of human lung cells exposed to α-particles. The NHBE cells from a nonsmoking, 15 yr-old male were purchased from Clonetics, San Diego, CA. The cells were grown on a 1.5 μm thick Mylar® film and exposed to α-particles from a stainless steel disk electroplated with ^{238}Pu. The total dose was either 2 or 4 Gy, delivered over 2 1/2 wk in six equal fractions. One wk after the final exposure, foci of phenotypically altered cells (PAC)s were detected in 40 of 64 (64%) of the 4 Gy cultures, and in 14 of 68 (21%) of the 2 Gy cultures. Foci of PACs were serially passaged as they became confluent. Samples of cells from passage three through six were fixed in Shandon's Cytospin Collection Fluid®, stained with propidium iodide, and assayed for cells exhibiting >5% binucleation or >5 micronuclei/1000 cells. Samples were scored blind. The results showed evidence of sustained genomic instability for as many as 45 cell divisions post irradiation in 6% of the PAC clones. The fact that elevated levels of binucleated and micronucleated cells were detected in the extended progeny of some NHBE cells exposed to α-particles indicates that these chromosomal aberrations were not a direct consequence of damage induced by ionizing radiation, but of sustained genomic instability that can develop early in the carcinogenesis process. [Research supported by U.S. DOE/OHER under contract No. DE-AC04-76EV01013].

A5-308 ALTERATIONS IN COAGULANT ACTIVITY FOLLOWING EXPOSURE TO INDUSTRIAL SOLVENTS: A POSSIBLE MARKER OF TOXIC CHEMICAL INDUCED PATHOGENESIS. Charles Lox, Dept. Obst.Gynecol. Texas Tech Univ. School Med. Lubbock, Tx 79430.

Male rats were exposed to 1 ppm of either benzene, carbon tetrachloride, benzaldehyde, or cyclohexanol in drinking water for 60 days. No adverse effect on body weight was noted, nor decreased consumption of water. Liver histology was not altered. Neither the platelet count or hematocrit were influenced. Specific coagulation factor activity was minimally altered if at all, including the hepatic synthesized coagulation factors; with one exception, factor VIII activity was markedly prolonged in clotting time (decreased activity). This corresponds to the prolonged APTT. This data suggests that factor VIII and the APTT (intrinsic pathway) alterations might indicate sub-cellular molecular pathogenesis following exposure to certain toxic chemicals.

A5-309 BIOLOGICAL SIGNIFICANCE OF EPOXIDE-INDUCED 3-HYDROXYALKYL-DEOXYURIDINE LESIONS IN DNA. Jerome J. Solomon, Cong Lai and Opinder S. Bhanot, Department of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, NY 10016

We are studying the biological significance of a new class of alkylpyrimidines, 3-hydroxyalkyl-deoxyuridine (3-HA-dU), produced by mutagenic and carcinogenic aliphatic epoxides. 3-HA-dU is formed in DNA after initial alkylation at N3 of dC followed by rapid hydrolytic deamination. Based on the facile (60-fold faster) deamination of ethylene oxide (EO)-induced 3-hydroxyyethyl-deoxycytidine (3-HE-dC) as compared to 3-ethyl-deoxycytidine, we hypothesized that the hydroxyl group at the C-2 position on the alkyl side chain of the 3-HE-dC intermediate facilitated hydrolytic deamination and have proposed a mechanism. Confirmation of this mechanism was achieved by isolation and mass spectral characterization of the cyclic intermediate. The significance of formation of 3-HA-dU in DNA was investigated by *in vitro* DNA replication of 3-HE-dU and propylene oxide (PO)-induced 3-hydroxypropyl-deoxyuridine (3-HP-dU). These lesions placed site-specifically in synthetic DNA templates blocked *in vitro* DNA replication, implicating these lesions in cytotoxicity of aliphatic epoxides. Under conditions of relaxed fidelity, i.e. absence of proofreading or substitution of the natural metal ion cofactor Mg^{++} with Mn^{++}, DNA synthesis past 3-HE-dU and 3-HP-dU occurred. During bypass synthesis in the absence of proofreading, dA and dT were incorporated opposite both 3-HE-dU and 3-HP-dU. Since 3-HA-dU is derived from epoxide-induced 3-HA-dC, incorporation of dA and dT opposite 3-HE-dU and 3-HP-dU implicates these lesions in G•C→A•T and G•C→T•A mutagenesis. Our studies suggest that 3-HE-dU and 3-HP-dU adducts may be critical premutagenic lesions produced by EO and PO respectively *in vivo*, and that 3-HA-dU may contribute to mutagenesis and initiation of cancer by other environmentally important epoxides. Supported by research grants from NIEHS (ES05694) and the Center for Indoor Air Research (CIAR 90-12) and NIH Center grants ES00260 and CA16087.

A5-310 **CHEMOPREVENTIVE EFFECT OF DIETARY BROCCOLI ON CARCINOGENESIS,** Ole Vang, Ole Andersen and Hilmer Sørensen[a], Dept. Life Sci. and Chem., Roskilde Univ., DK-4000 Roskilde, Denmark; [a]Chem. Dept., Royal Veterinary Univ., DK-1791 Frederiksberg, Denmark.

Epidemiological studies indicate that fresh fruit and vegetables reduce the risk of cancer. Several plant chemicals have been proposed to act anti-carcinogenic, but the levels of these substances are low in the human diet. Therefore, we propose that **a)** several anti-carcinogenic substances act additively and **b)** individual substances act on different steps in carcinogenesis.

A research programme has been initiated to investigate the different anti-carcinogenic effects of **1)** indole-3-carbinol and **2)** the complex mixture, broccoli. Indole-3-carbinole is formed during degradation of glucobrassicin, found in broccoli.

1) Formation of different condensation products of indole-3-carbinol at near neutral pH was analyzed by capillary electrophoresis. The modulating effect of these products on several cytochrome P-450 enzymes, hormone and carcinogen metabolism in liver, kidney and colon was studied in the rat. Di-, tri- and tetrameric-products of indole-3-carbinol were formed having modulating effect on CYP1A1, 1A2 and 2B1 enzymes.

2) In previous reports of the modulating effect of *brassica* vegetables on carcinogenesis, no correlations have been made to the content of relevant anti-carcinogenic compounds. The actual levels of different anti-carcinogens (glucosinolates, flavonoid-glucosides and S-containing amino acids) were determined in different sources of broccoli, and we may correlate the response of dietary broccoli to the content of anti-carcinogens. The contents vary dramatically with the species of broccoli and by addition of fertilizer.

A5-311 GLUTATHIONE AND FREE AMINO ACIDS FORM STABLE ADDUCTS WITH DNA FOLLOWING EXPOSURE OF CHO CELLS TO CHROMATE, Anatoly Zhitkovich, Victoria Voitkun and Max Costa, Institute of Environmental Medicine, New York University Medical Center, New York, NY 10016

Exposure of cells to carcinogenic Cr(VI) compounds has been known to result in the formation of several types of DNA lesions such as strand breaks, DNA-protein crosslinks, as well as abundant non-characterized Cr-DNA adducts. Hexavalent chromium compounds have been shown to be mutagenic in most bacterial and eukariotic test systems although the nature of DNA modifications underlying the chromium mutagenicity are not known. We found that exposure of CHO cells to potassium chromate led to the formation of stable DNA adducts involving intracellular amino acids and glutathione. Cysteine, glutamic acid, and histidine are the major DNA-crosslinked amino acids in chromate-treated cells. EDTA was found to effectively dissociate the DNA-amino acid/glutathione crosslinks indicating that Cr(III) directly participated in the formation of these adducts. The DNA-associated amino acids did not originate from DNA-protein crosslinks, but rather represented complexed free amino acids. The crosslinks of glutathione and amino acids were estimated to account for as much as 50% of DNA-bound chromium following exposure to mutagenic chromate concentrations and, therefore, this type of DNA lesions constitute the major form of Cr-DNA adducts.

A5-312 ASSESSMENT OF THE EFFECT OF ENVIRONMENTAL VANADIUM UPON PROTEIN PHOSPHATASES PARTICIPATING IN CELL DIVISION PROCESSES, Henry K. Parsadanian, Svetlana N. Marchenko, Igor R. Barilyak, Laboratory of Biochemistry, Ukrainian Scientific Centre of Hygiene, Kiev-253660, Ukraine

Vanadium is one of the enough distributed elements obtained both in nature and in the products of human industrial and economic activities. In Ukraine it is concentrated in regions of developed metallurgic industry, in big urban areas etc. The biological and toxic functions of vanadium are now actively discussed. Vanadate is thought to mimic the effects of insuline and some growth factors whose receptors have tyrosine kinase activity, and stimulates the tyrosine kinase activity of $pp60^{v\text{-}src}$ in Rous sarcoma virus-transformed cells. To rationalize the effect of vanadium compounds mentioned above we proposed a hypothesis that these effects are able to be realized not only via tyrosine kinase stimulation, but also by the regulation of protein (tyrosine) phosphatase (TPP). The *in vitro* effect of various concentration of ammonium meta-vanadate upon the TPP activity of rat tissues has been examined. To reveal the TPP activity casein was used as a substrate. The sensitivity of separate regions of CNS to vanadate was different. Possessing the highest TPP activity, hemispheres were the most sensitive to vanadate, and lost more than 40% of the initial activity when the concentration of vanadate reached in the incubation media 10 μM. At 1 mM concentration of vanadate 2/3 of the initial activity disappeared. Medulla oblongata and cerebellum TPP in a less measure suffered from vanadate. In more "ancient" structures of spinal cord low concentrations of vanadate (0.1 - 1μM) stimulated more, than two-fold the activity of the enzyme. Only at mM concentrations of vanadate the activity of the phosphatase was undoubtely decreased. One may speculate that the effect obtained is a summary one, and the less sensitive protein phosphatases are mostly represented in ancient regions of CNS. Among the other TPPs studied, the kidney enzyme was the most stable to the vanadate action. The pronounced inhibitory effect revealed in experiments with heart tissue TPP activity. The trend to decrease was seen in the presence of 1 μM and the inhibition reached 60% at the presence of 0.1 mM vanadate. Our data demonstrate that the effects of vanadate upon the processes being under the control of protein phosphorylation-dephosphorylation (microtubules dissociation and disorganization, transcribtion and translation) can be realized not only by activation of kinase reaction but also via the inhibition of protein phosphatases.