Overview

The Division of Intramural Research of the National Institute of Environmental Health Sciences (NIEHS) welcomes applications for 12 month Fellowships in Environmental Medicine for Medical Students. The NIEHS, as part of the National Institutes of Health (NIH), is dedicated to reducing the burden of environmentally associated diseases and dysfunctions on human health. This mission is achieved by conducting and supporting basic and applied research on how environmental exposures affect biological systems and human health, on the identification of susceptible subpopulations, and on the interaction between the environment, genetics, and age.

The purpose of the Division of Intramural Research of the NIEHS is to provide high quality research of relevance to the Institute's mission. Intramural research at the NIEHS is organized into three highly interrelated, interactive, and synergistic programs that highlight the three areas of research excellence of NIEHS. These programs focus on: **basic research**, which contributes to the basic understanding of biological and chemical processes; **environmental toxicology**, dedicated to the understanding of the contributions of environmental agents to human disease and dysfunction; and **environmental disease and medicine**, which focuses on underlying mechanisms of environmentally associated diseases.

Intramural research scientists are highly interactive and are often engaged in interdisciplinary research, which encourages efficient testing of novel ideas, innovative hypotheses, and new paradigms. These interactions are promoted by the housing of the entire Intramural Program in a single building and by seminars, research faculties, and symposia that cut across traditional laboratory boundaries. NIEHS scientists are also actively involved in translational and clinical research. New advances in cell and molecular biology are being extended not only into molecular medicine (from bench to bedside) but also into disease prevention (from bench to longer, healthier lives).

Fellowships in Environmental Medicine for Medical Students provide approved students with an annual stipend of approximately $18,000 (depending on experience) to work full-time for one year with NIEHS scientists. The NIEHS cannot pay for medical school tuition. Short research profiles of NIEHS scientists who are available to serve as sponsors for these fellows are provided in this booklet. The NIEHS also accepts into its laboratories students already funded by such agencies as the Howard Hughes Medical Institute and the Stead Foundation.

Application materials must generally be received by **January 31** except under unusual circumstances. Questions about the application deadline should be directed to Dr. Steven Akiyama at akiyama@niehs.nih.gov or to Dr. Perry Blackshear at black009@niehs.nih.gov. Applicants will usually be notified of the Scientific Director's decision no later than approximately **March 1**. Laboratory research can generally start

any time between July 1 and September 1 of the same year in which the application is submitted.

Application Procedure

- Identify one or more possible sponsors from the research profiles provided in this booklet.

- Contact potential sponsors directly to make sure these individuals have space in their laboratory for the upcoming year and to arrange to discuss possible research projects. Telephone numbers and e-mail addresses for all eligible sponsors are provided with the research profiles. It is also advisable to visit the DIR laboratories and meet with prospective sponsors / mentors.

- In consultation with the prospective sponsor, prepare a one- to two-page description of the proposed research project.

- Compose a brief (approximately one paragraph) statement of career goals

- Obtain three letters of recommendation that can help the NIEHS review panel evaluate your suitability for a full-time research training position.

- Submit an application package consisting of
  A cover letter
  Your curriculum vitae
  Medical School transcripts
  The statement of research career goals
  Three confidential letters of recommendation
  The short research project description

- Send applications and letters of recommendation to:

  Dr. Seven K. Akiyama
  Medical Student Training Program
  National Institute of Environmental Health Sciences
  PO Box 12233, Mail Drop A2-09
  111 T.W. Alexander Drive
  Research Triangle Park, NC 27709

Application materials must be received by January 31.

Genetics

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Unlike many toxicity endpoints, the deleterious effects of mutations are difficult or impossible to relieve and, when arising in germ cells, will persist into subsequent generations. Thus, mutation prevention is a more hopeful strategy. To this end, our research is aimed at uncovering the mechanisms by which organisms generate mutations. Specifically, we use the bacterium *Escherichia coli* as a model system for a thorough dissection of the pathways of mutation production and mutation prevention.

Mutator mutants (with a higher mutation rate than the wild-type strain) provide detailed insights into mechanisms that organisms use to prevent mutations. These mechanisms include base selection by the DNA polymerase, exonucleolytic, and DNA mismatch correction, as well as numerous DNA repair systems not directly linked to DNA replication.

On the other hand, antimutator mutants (with a lower mutation rate than the wild-type) provide insights into the origins of mutations that normally escape correction, notably the background of spontaneous mutations. We are searching for antimutators that act through well defined pathways in order to understand the precise factors responsible for spontaneous mutations. As a first step, we have isolated antimutator strains that replicate their DNA with increased accuracy and we are using these strains to determine what fraction of spontaneous mutations is due to uncorrected replication errors.

The precise mechanisms by which *E. coli* achieves high fidelity of chromosomal DNA replication are also investigated through *in vitro* DNA replication studies using purified pol III holoenzyme (HE) and its reconstituted subassemblies. A comparison of *in vivo* and *in vitro* polymerase errors in the same sequence target (the *lacI* gene) has revealed pronounced differences between the two, suggesting that additional factors contribute to *in vivo* replication fidelity other than polymerase base selection and proofreading. To investigate these questions we are exploring the possible role of the pol III accessory factors in chromosomal replication fidelity. In particular, the dnaX gene, encoding both the gamma and tau subunits of HE, appears to play an important role as evidenced by a newly discovered mutator phenotype associated with certain dnaX mutants.

We are also investigating whether the enzymatically distinct leading and lagging strand replication machineries that operate the replication fork have distinct fidelities. Current data suggest that this is indeed the case, and we are investigating the underlying mechanisms by genetic and biochemical means.

Relevant Publications:

Taft-Benz, S. A. and Schaaper, R. M. (1999) The C-terminal domain of DnaQ contains the polymerase binding site. J. Bacteriol. **181**: 2963-2965.

Fijalkowska, I.J., Jonczyk, P., Tkaczyk, M., Bialoskorska, M., and Schaaper, R.M.(1998) Unequal fidelity of leading strand and lagging strand DNA replication on the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. USA 95: 10020-10025.

Pham, P.T., Olson, M.W., McHenry, C.S., and Schaaper, R.M. (1998) The basesubstitution and frameshift fidelity of Escherichia coli DNA polymerase IIIholoenzyme in vitro. J. Biol. Chem. 273: 23575-23584.

Taft-Benz, S.A., and Schaaper, R.M. (1998) Mutational analysis of the 3'->5'proofreading exonuclease of *Escherichia coli* DNA polymerase III. Nucleic Acids Res. 26: 4005-4011.

Fijalkowska, I.J., and Schaaper, R.M. (1996) Mutants in the Exo I motif of *E. coli*dnaQ: defective proofreading and inviability due to error catastrophe. Proc. Natl. Acad. Sci. USA 93: 2856-2861.

Fijalkowska, I.J., Dunn, R.L., and Schaaper, R.M. (1993) Mutants of E*scherichiacoli* with increased fidelity of DNA replication. Genetics 134: 1023-1030.

Schaaper, R. M. (1993) Base selection, proofreading and mismatch repair during DNA replication in *Escherichia coli*. J. Biol. Chem. 268: 23762-23765.

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The six billion nucleotides of the diploid human genome are replicated in only a few hours in a process generating so few errors that the spontaneous mutation rate may be much less than 1 mutation per genome per cell division. Three steps are responsible for this high replication fidelity: base selectivity and xonucleolytic proofreading of errors during DNA polymerization, and correction of errors afterwards.

The research in this laboratory is intended to further our understanding of these processes and how their failure or perturbation yields mutations. Our primary experimental approach is to study the fidelity of DNA synthesis in vitro. This includes analyzing reactions in which single-stranded DNA is replicated by DNA polymerases, especially those that replicate eukaryotic chromosomal DNA or the HIV-1 genome. Of particular interest is the relationship between replication fidelity and the structure of DNA polymerases as determined by X-ray crystallography. We are also investigating the fidelity of replication of double-stranded DNA catalyzed by the multiprotein replication apparatus of human cells, using either undamaged DNA or substrates containing adducts of known carcinogens.

Finally, we are examining the mechanisms and gene products that correct single-base mispairs and loops resulting from strand misalignments. Failure to perform mismatch repair accurately and efficiently leads to cell death and to various forms of genome instability, the consequences of which include cancer, heart disease, heritable birth defects, and perhaps aging.

Relevant Publications:

Drotschmann, K., Clark, A.B., Tran, H.T., Gordenin, D.A., Resnick, M.A. and Kunkel, T.A. (1999) Mutator phenotypes of yeast strains heterozygous for mutations in the *MSH2* gene. *Proc. Natl. Acad. Sci. USA* **96**, 2970-2975

Shcherbakova, P. and Kunkel, T.A. (1999) Mutator phenotypes conferred by *MLH1* overexpression and by heterozygosity for *mlh1* mutations. *Molec.Cell. Biol.* **19**, 3177-3183.

Osheroff, W. P., Beard, W.A., Wilson, S.H. and Kunkel, T.A. (1999) Base substitiution specificity of DNA polymerase β depends on interactions in the DNA minor groove. *J. Biol. Chem.*, **274**, 20749-20752.

Kroutil, L.C. and Kunkel, T.A. (1999) Strand slippage during replication of CAG repeat sequences by DNA polymerases. *Nucl. Acids Res.* **27**, 3481-3486
Drotschmann, K., Clark, A.B. and Kunkel, T.A. (1999) Mutator phenotypes of common polymorphisms and missense mutations in MSH2. *Current Biology* **9**, 907-910

Lewis, D.A., Bebenek, K., Beard, W.A., Wilson, S.H. and Kunkel, T.A. (1999) Altered DNA replication fidelity conferred by an amino acid change in the nucleotide binding pocket of HIV-1 reverse transcriptase. *J. Biol. Chem.* **274**, 32924-32930.

Edelmann, W., Umar, A., Yang, K., Heyer, J., Kucherlapati, M., Lia, M., Kneitz, B., Avdievich, E., Fan, K., Wong, E., Crouse, G., Kunkel, T., Lipkin, M., Kolodner, R.D. and Kucherlapati, R. (2000) The DNA mismatch repair genes *Msh3* and *Msh6* cooperate in intestinal tumor suppression. *Cancer Res.* **60**, 803-807.

Wilson, S. H. and Kunkel, T.A. (2000) Passing the baton in base excision repair. *Nature Struct. Biol.* **7**, 176-178.

Matsuda, T., Bebenek, K., Masutani, C., Hanaoka, F. and Kunkel, T.A. (2000) Low fidelity DNA synthesis by human DNA polymerase-eta. *Nature* **404**, 1011-1013.

Osheroff, W.P., Beard, W.A., Yin, S., Wilson, S.H. and Kunkel, T.A. (2000) Minor groove interactions at the DNA polymerase active site modulate single-base deletion errors. *J. Biol. Chem.*, in press.

Kunkel, T.A. and Bebenek, K. (2000) DNA replication fidelity. *Annu. Rev. Biochem.* 69, in press.

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We investigate the genetics and enzymology of DNA repair and mutation in the classical model system bacteriophage T4 which, together with Escherichia coli, has yielded most of our understanding of the molecular basis of mutation. We also investigate global aspects of spontaneous mutation rates.

The main determinant of the T4 mutation rate is its DNA polymerase and associated proofreading exonuclease. This enzyme maintains replication fidelity largely independently of its interactions with the other accessory proteins and enzymes of DNA replication. Using both T4 and the related phage RB69, we are probing the relationships between polymerase structure and replication fidelity both in vivo and in vitro.

Nonlethal mutations in genes encoding certain enzymes of DNA replication render T4 generally sensitive to DNA damage. We are investigating this "replication repair" process using both genetic and enzymological approaches.

Rates of spontaneous mutation fall into a few distinct categories that probably represent evolutionary balances between the deleterious consequences of most mutations and the investments required to further reduce mutation rates. All DNA-based microbes examined produce about one mutation per 300 chromosome replications. Higher eukaryotes have the same or a slightly higher rate, so that rates per sexual generation are close to the maximum compatible with life, about one mutation per gamete. RNA viruses have mutation rates of about one per genome per chromosome replication, and small increases in their mutation rates are lethal.

Relevant Publications:

Bebenek, A., Smith, L.A., and Drake, J.W. (1999) Bacteriophage T4 *rnh* (ribonuclease H) null mutations: effects on spontaneous mutation and epistatic interaction with *rII* mutatins. J. Bacteriol. 181: 3123-3128.

Drake, J.W., and Holland, J.J. (1999) Mutation rates among lytic RNA viruses. Proc. Natl. Acad. Sci. USA 96: 13910-13913.

Epidemiology

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Environmental toxins are known to cause infertility, fetal loss and malformations in laboratory animals, but these effects have not been well studied in humans. Dr. Wilcox's work has been to extend the study of environmental exposures into the area of human reproduction.

Dr. Wilcox's work falls into three areas. The first is conception and early pregnancy. Dr. Wilcox and his research group have shown that at least one-quarter of human pregnancies end in loss before the woman is aware she is pregnant (1988). Their methods for measuring early pregnancy loss have been widely adapted by environmental researchers searching for subtle effects of reproductive toxins. They were also the first to use biochemical markers of ovulation to determine a woman's fertile window. Among healthy women trying to conceive, there is an average of six fertile days in each menstrual cycle, ending on day of ovulation (1995a). Further, in pursuing causes of fertility and infertility they have shown that men prenatally exposed to high doses of estrogen (diethylstilbestrol) are not impaired in their own fertility (1995b).

His second area of interest is birthweight. Low birthweight is a convenient endpoint in studies of environmental toxins. However, low birthweight may not be on the causal pathway to perinatal risk, as most people assume. Dr. Wilcox developed this idea in a series of papers suggesting an alternative approach to the analysis of birthweight. He and his colleagues recently showed that the relatively high rate of infant mortality in the US compared to Norway is not due to the smaller size of US infants, but to the higher rates of preterm delivery in the US -- a public health problem that is only recently being recognized (1995c).

Wilcox's third area of research is the role of genetic susceptibility to environmental teratogens. Both genetic and environmental factors contribute to the risk of birth defects (1994). He and his colleagues have begun a case-control study of facial clefts in which they will test the hypothesis that the A2 allele of TGF-alpha (transforming growth factor) represents a susceptible genotype for the teratogenic effects of maternal smoking and perhaps other toxicants. A second hypothesis is that folic acid deficiency increases the risk of facial clefts, particularly within a genetically susceptible subgroup defined by an allele of the gene controlling the enzyme MTHFR.

Relevant Publications:

Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, Armstrong EG, Nisula BC: Incidence of early loss of pregnancy. New Engl J Med 319:189-94, 1988.

Lie RT, Wilcox AJ, Skjaerven R: A population-based study of risk of recurrence of birth defects. New Engl J Med 331:1-4; 1994.

Wilcox AJ, Weinberg CR, Baird DD. Timing of sexual intercourse in relation to ovulation: Effects on the probability of conception, survival of the pregnancy and sex of the baby. New Engl J Med 333: 1517-21, 1995a.

Wilcox AJ, Baird DD, Weinberg CR, Hornsby PP, Herbst AL: Fertility in men exposed prenatally to diethylstilbestrol. New Engl J Med 332:1411-16; 1995b.

Wilcox AJ, Skjaerven R, Buekens P, Kiely J: Birth weight and perinatal mortality: A comparison of the United States and Norway. JAMA 273:709-11; 1995c.

Wilcox AJ, Weinberg CR, Lie RT: Distinguishing the effects of maternal and offspring genes through studies of "case-parent triads." American J Epidemiol 148: 893-901, 1998.

Skjaerven R, Wilcox AJ, Lie RT. A population-based study of survival and childbearing among female subjects with birth defects and the risk of recurrence in their children. New Engl J Med 340: 1057-1062, 1999.

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Dr. Sandler's research focuses on environmental causes of chronic disease in adults, in particular, studies of risk factors for leukemia and myelodysplasia, health effects of residential and occupational exposure to radon, and the health consequences of exposure to agricultural chemicals. Other ongoing research focuses on risk factors for chronic kidney disease and on health effects of exposure to environmental tobacco smoke. Dr. Sandler is developing a large prospective study of breast cancer risk among women who have had a sister with breast cancer. This study will afford many opportunities to examine environmental and genetic risk factors for breast cancer and other diseases in women.

The failure of previous studies to identify strong risk factors for leukemia may be due, in part, to poor characterization of leukemia subtypes. Dr. Sandler's studies have explored the role of clonal chromosome abnormalities detected in the bone marrow of patients, oncogenes, and polymorphisms in genes that affect the metabolism of potential carcinogens. They showed that persons with the null genotype for glutathione-S-transferase theta (GSTT1) are at greatly increased risk for myelodysplasia and that leukemia patients with ras-gene mutations are more likely to have been exposed to solvents. They further demonstrated links between smoking and other exposures and specific chromosome abnormalities in AML and ALL, although differences were not as great as expected. They also demonstrated that myelodysplasia may be a marker for chemical exposure in myeloid leukemia. Current efforts are exploring the role of family history of cancer in leukemia risk and prognosis.

The radon study was motivated by widespread interest in the possibility that indoor radon exposure is a major cause of lung cancer in the US. The study, based in Utah and Connecticut, involves 1,474 lung cancer patients and 1,811 population controls for whom were obtained detailed exposure histories and measured radon levels in current and past homes. Data collection is complete, and evaluation of risk factors is under way. Current efforts focus on the role of radon, environmental tobacco smoke, particulate matter air pollution, and arsenic in lung cancer risk.

Another study is of cancer incidence in a cohort of 18,000 Czech uranium miners who are exposed to radon. Preliminary results suggest increased risk for cancers in addition to lung cancer, including cancer of the larynx, gastric cancer, and leukemia. Case-cohort

studies will explore the influence of additional exposures including dusts and cigarette smoking in addition to radon exposure.

Dr. Sandler is collaborating with the NCI and EPA on a prospective study of cancer risk in a cohort of nearly 75,000 licensed pesticide applicators and spouses. In this study, Dr. Sandler's efforts are focused on non-cancer outcomes including reproductive health, respiratory disease, immune function, and kidney disease. An important feature of this study is the comprehensive exposure and biologic monitoring for a sample of the cohort and the opportunity for long-term follow-up of the cohort. Currently, five-year follow-up interviews are being conducted with pesticide applicators and spouses. Data are now being linked with data from cancer registries and state and national vital statistics to identify deaths and incident cancers among cohort members.

Relevant Publications:

Sandler DP, Smith JC, Weinberg CR, Buckalew VM, Dennis V, Blythe W, Burgess WP: Analgesic use and chronic renal disease. New Engl J Med 320: 1238-43, 1989.

Sandler DP, Helsing KJ, Comstock GW, Shore DL: Factors associated with past household exposure to tobacco smoke. Am J Epidemiol 129: 380-387, 1989.

Sandler DP, Burr FR, Weinberg CR: Nonsteroidal anti-inflammatory drugs and risk of chronic renal disease. Annals Int Med 115: 165-172, 1991.

Weinberg CR, Sandler DP: Randomized recruitment in case-control studies. Am J Epidemiol 134: 421-432, 1991.

Taylor JA, Sandler DP, Bloomfield CD, Shore DL, Ball ED, Neubauer A, McIntyre OR, Liu E: ras oncogene activation and occupational exposures in AML. J Natl Cancer Inst 84: 1626-1632, 1992.

Sandler DP, Shore DL, Anderson JR, Davey FR, Silver RT, Aisner J, Canellos GP, Weiss RB, Trump DL, Arthur D, Wurster-Hill D, McIntyre OR, Bloomfield CD: Cigarette smoking and risk of acute leukemia: Associations with morphology and cytogenetic abnormalities in bone marrow. J Natl Cancer Inst 85: 1994-2003, 1993.

Chen H, Sandler DP, Taylor JA, Shore DL, Liu E, Bloomfield CD, Bell DA: Increased risk for myelodysplastic syndromes among those with glutathione transferase theta 1 (GSTT1) gene defect. Lancet 347: 295-297, 1996.

Alavanja MCR, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF,

Pennybacker M, Rothman N, Dosemeci M, Blair A: The Agricultural Health Study. Environ Health Perspect 104: 362-369, 1996.

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The development of most cancers and chronic diseases is a multifactorial process involving issues of susceptibility (from genetic or nutritional factors) and environmental exposures. Males and females may respond differently to some environmental exposures, and this variability may reflect hormonal influences on disease susceptibility and etiology. Dr. Cooper's research focuses on these complex interactions.

Ovarian function has direct and indirect consequences for women's health since, in women, the production of estrogen and progesterone is controlled by the ovary. Menopause (or "ovarian failure") represents a normal aspect of aging, but it also influences risk for a wide variety of diseases. Understanding the factors that influence follicular atresia may provide insights into apoptosis, the aging process, and disease risk. Osteoporosis and some autoimmune conditions are examples of hormonally-mediated diseases that occur in either gender, but are more common in women.

Ovarian function may be viewed as an outcome in itself, and as a factor that interacts with a broad array of environmental exposures and genetic characteristics to influence the development of chronic diseases. This broad view of the multiple factors involved in disease pathogenesis is necessary in order to understand fully the mechanisms through which hormones affect disease risk, and to develop approaches to decrease the incidence of diseases which differentially affect women. Exposures that either mimic or modulate hormones are particularly relevant to the mission of NIEHS, and Dr. Cooper's work incorporates this interest.

Relevant Publications:

Cooper GS, Hulka BS, Baird DD, Savitz DA, Hughes CL, Weinberg CR, Coleman RA, Shields JM. Galactose consumption, metabolism, and gonadotropin levels in women of late reproductive age. Fertil Steril 62:1168-75; 1994.

Cooper GS, Baird DD. The use of questionnaire data to classify peri- and pre-menopausal status. Epidemiology 6: 625-8; 1995.

Cooper GS, Baird DD, Hulka BS, Weinberg CR, Savitz DA. Hughes CL. FSH concentrations in relation to active and passive smoking. Obstet Gynecol 85: 407-11; 1995.

Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Min Res 11: 1841-1849; 1996.

Cooper GS, Sandler DP, Whelan EA, Smith KR. Association of physical and behavioral characteristics with menstrual cycle patterns in women age 29 to 31. Epidemiology 7: 624-628; 1996.

Cooper GS, Sandler DP. Long-term effects of reproductive-age menstrual cycle patterns on peri- and post-menopausal fracture risk. Am J Epidemiol; 145: 804-809.

Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Parks CG, Gilkeson GS: Hormonal, environmental, and infectious disease risk factors for the development of systemic lupus erythematosus. Arthritis & Rheumatisium 41:1714-1724, 1998.

Cooper GS, Miller FW, Pandey JP. The Role of Genetic Factors in Autoimmune Disease: Implications for Environmental Research. Environ Health Perspect, 1999; 107 Suppl 5:693 700.

Cooper GS, Thorp JM. FSH Levels in relation to hysterectomy and unilateral oophorectomy. Obstet Gynecol, 1999; 94:969-972.

Cooper GS, Baird DD, Weinberg CR, Ephross SA, Sandler DP. Age at menopause and childbearing patterns in relation to mortality. Am J Epidemiol, 2000; 151:620-623.

Neurosciences

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Research Summary:

We use the patch clamp technique to study the signal transduction pathways that regulate the excitability of mammalian cells by controlling ion channel activity. In particular many neurotransmitters and hormones inhibit excitability by stimulating potassium channels through serine/threonine protein phosphatases, which are the targets of a growing list of the most potent and widespread aquatic microbial toxins. We have cloned some of these channels and the enzymes that regulate them in order to confirm our conclusions from physiological/pharmacological studies at the molecular level.

Projects are available using patch clamp and/or molecular biology to answer the following questions in cardiac, endocrine, hematopoietic or neuronal cells: How are potassium channels regulated by reversible protein phosphorylation? What molecules link G protein-coupled receptors to potassium channel stimulation? How are these pathways involved in degenerative diseases of the aging nervous system?

Relevant Publications:

Wang, D. & Armstrong, DL (2000) Tetraethylammonium potentiates the activity of muscarinic potassium channels in guinea pig atrial myocytes. *J. Physiol.*, in press.

Hall, SK & Armstrong, DL (2000) Conditional and unconditional inhibition of calcium-activated potassium channels by reversible protein phosphorylation. *J. Biol. Chem.*, **275**, 3749-3754.

Armstrong, DL & Rossie, S., eds. (1999) Ion Channel Regulation. *Adv. Second Messenger & Phosphoprotein Research*, Vol. 33, Academic Press, San Diego.

Skinner, J., Sinclair, C., Romeo, C., Armstrong, D., Charbonneau, H. and Rossie, S. (1997) Purification of a fatty acid-stimulated protein-serine/threonine phosphatase from bovine brain and its identification as a homolog of protein phosphatase 5. *J. Biol. Chem*. **272** (36): pp 22464-22471.

Duerson, K., White, R.E., Jiang, F., Schonbrunn, A. and Armstrong, D.L., (1996) Somatostatin stimulates BK_{Ca} channels in rat pituitary tumor cells through lipoxygenase metabolites of arachidonic acid. *Neuropharmacology* **35**: 949-961.

Shipston, M.J. and Armstrong, D.L. (1996) Activation of protein kinase C inhibits calcium-activated potassium channels in rat pituitary tumor cells. *J. Physiol.* **493.3**: 665-672.

Armstrong, D.L. and White, R.E. (1994) Natriuretic peptides and receptors. *Scientific American Science & Medicine* **1**, 34-43.

Armstrong, D.L. and White, R.E. (1992) An enzymatic mechanism for potassium channel stimulation through pertussis toxin-sensitive G-proteins. *Trends Neurosci.*, **15**: 403-408.

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Neuronal communication between cells in the nervous system occurs at the synapse, where the release of neurotransmitter (NT) (e.g. serotonin [5-HT], acetylcholine [ACh], glutamate, or GABA) by the presynaptic terminal diffuses across the synaptic cleft and binds to and activates various ligand-gated ion channels on the postsynaptic membrane. Therefore ligand-gated ion channels mediate rapid (e.g., on the order of milliseconds) synaptic transmission, and changes in the function of these channels will have profound effects on neuronal excitability. As these channels are the targets of various environmental toxins and signal transduction cascades, which can regulate their function on a much longer time scale (e.g., seconds, minutes, or even hours), these pathways can regulate to a large extent synaptic efficacy (i.e. the regulation of the strength of the synaptic connections between neurons) and plasticity. To better understand the basic mechanisms and regulation of synaptic transmission in the CNS, the lab is focusing on the function and regulation of the ligand-gated ion channels gated by acetylcholine (ACh; this is referred to as the nicotinic receptor as it is activated by nicotine) and serotonin (i.e., the 5-HT3 receptor) in the hippocampus. Hippocampal interneurons contain functional somato-dendritic nicotinic and 5-HT3 receptors, both receptors of which may be involved in cognitive processes. Hippocampal interneurons are inhibitory as they are known to release the inhibitory neurotransmitter GABA. Although there are many fewer interneurons than the numbers of principal excitatory cells, a single interneuron can innervate and regulate the activity of hundreds of excitatory cells in the hippocampus. Although the nicotinic and 5-HT3 receptor channels are known to be involved in a variety of physiological processes, the precise nature of these actions are not currently known, and is the major focus of investigation in the lab.

Relevant Publications:

Sudweeks, S, & Yakel, J.L. Functional and molecular characterization of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurones. J. Physiology, In Press.

Shao, Z. & Yakel, J.L. Single channel properties of neuronal nicotinic ACh receptors in stratum radiatum interneurons of rat hippocampal slices. J. Physiology, In Press.

Jones, S., Sudweeks, S. & Yakel, J.L. (2000) Nicotinic receptors in the brain: correlating physiology with function. Trends in Neurosciences 22: 555-561.

Kriegler, S., Sudweeks, S, & Yakel, J.L. (1999) MTSEA potentiates 5-HT3 receptors containing the nicotinic a4 subunit. Neuropharmacology 38: 1913-1915.

Kriegler, S., Sudweeks, S, & Yakel, J.L. (1999) The nicotinic a4 receptor subunit contributes to the lining of the ion channel pore when expressed with the 5-HT3 receptor subunit. J. Biol. Chem. 274: 3934-3936.

Van Hooft, J.A., Spier, A.D., Yakel, J.L., Lummis, S.C.R., & Vijverberg, H.P.M. (1998) Promiscuous coassembly of serotonin 5-HT3 and nicotinic a4 receptor subunits into Ca2+ permeable ion channels. Proc. Natl. Acad. Sci. USA 95: 11456-11461.

Jones, S., & Yakel, J.L. (1998) Ca2+ influx through voltage-gated Ca2+ channels regulates 5-HT3 receptor channel desensitization in rat glioma X mouse neuroblastoma hybrid NG108-15 cells. J. Physiol. 510: 361-370

Jones, S., & Yakel, J.L. (1997) Functional nicotinic ACh receptors on interneurones in the rat hippocampus. J. Physiol. 504: 603-610.

Zhu, Y., & Yakel, J.L. (1997) Calcineurin modulates G protein-mediated inhibition of N-type calcium channels in rat sympathetic neurons. J. Neurophysiol. 78: 1161-1165.

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Pathology

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Research interests have focused on the biology and pathogenesis of rodent hepatic and pulmonary toxicity and carcinogenesis and on the application of new technologies such as molecular pathology and magnetic resonance imaging to these areas. There is a strong emphasis on immunohistochemistry and image analysis and recent efforts have utilized magnetic resonance microscopy as a research tool in collaboration with Duke's Department of Radiology. Techniques utilized include quantitative stereology of altered hepatic foci, various measures of cell proliferation in tissues of treated rodents, nonisotopic in situ hybridization, laser capture microscopy, and tissue microarrays. Current research emphasis is on prostate cancer utilizing transgenic mice.

Relevant Publications:

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Women have a distinct profile of diseases over their lifespan. The Laboratory of Women's Health seeks to determine how environmental toxins and stresses shape this profile. Diseases under study include breast cancer, ovarian cancer, uterine leiomyoma, ovarian dysfunction, pregnancy and parturition dysfunctions, and autoimmune diseases. The Laboratory is particularly concerned with developing and using genetically defined animal models and translating findings between animal and human studies. A focus is to identify key genes and signaling systems of the reproductive and immune systems in their interactions with the environment over time and lifestage. The ultimate goal is to reduce the burden of environmentally related diseases in women by integrating the disciplines of genetics, endocrinology, immunology, pathology and epidemiology.

Reproductive Pathology Group

Barbara J. Davis, VMD. Ph.D., Dipl. ACVP, Principal Investigator

The Female Reproductive Pathology Group studies the pathophysiology of chemically mediated ovarian and uterine dysfunction, and ovarian and uterine cancer in women and rodents. The overall goals of our research are to determine the role of key genes and signaling molecules in ovarian and uterine muscle cell growth, differentiation, and physiology, and understand how to modify these pathways to ameliorate dysfunction and cancer; and identify the target cells and biochemical and molecular mechanisms by which synthetic or naturally-occurring environmental chemicals cause ovarian dysfunction or ovarian cancer in in vivo and in vitro models. We have a particular interest in prostaglandin pathways in ovarian function, pregnancy and parturition and their relationship to tumors of the ovary and uterine fibroids.

Comparative Carcinogenesis Group

Roger Wiseman, Ph.D., Principal Investigator

The Comparative Carcinogenesis Group studies the BRCA1 and BRCA2 breast cancer susceptibility genes in humans and rodents. Since their identification by positional cloning, we have demonstrated that somatic mutations in these genes are extremely rare in sporadic human breast and ovarian cancers, in contrast to expectations based on other cancer susceptibility genes. Because greater than 90% of the ~500 BRCA1 and

BRCA2 mutations identified to date lead to the premature termination of protein synthesis, we have developed protein truncation assays as an efficient mutation screening technique for these genes. We have also characterized the rodent homologues of BRCA1 and BRCA2 which has helped identify potential functional domains of these proteins based on evolutionarily conservation. Current efforts are focused on the creation of mice with BRCA1 and BRCA2 defects through gene targeting in embryonic stem cells. Mouse models for defects in these genes should be useful for the development of cancer prevention and therapeutic strategies, as well as carcinogen risk assessment.

Biometry

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The Genetic Risk Group's (GRG) primary goal is to characterize the adverse health risk for human populations associated with exposure to chemicals, and also with genetic variability in the metabolism of these chemicals. Thus, GRG seeks to determine the risk associated with human exposure to carcinogens by investigating the link between environmental exposures, the resulting biological effects of the exposure (biomarkers), and the susceptibility factors that modulate this process. Specifically we are engaged in population-based molecular epidemiologic studies that investigate: 1) the relationship between exposure to chemicals and intermediate biomarkers of genetic damage, 2) the relationship between phenotypic polymorphism and genotype, 3) the role of metabolism polymorphism in modulating exposure-related genetic damage, 4) the role of genetic polymorphism in modulating exposure-related risk of cancer. A major interest is in developing new molecular markers of risk, using these markers in multiendpoint molecular epidemiology studies, and incorporating data from our studies into risk assessment models.

We have recently observed that individuals with "at risk" N-acetyltransferase 1 genotypes (NAT1*10) have 2-fold higher levels of DNA adducts in bladder tissue, are at 2.8 to 26 fold increased risk of bladder cancer, and have increased risk for gastric and colorectal cancer. These data suggest that NAT1 genotype may be an important new genetic risk factor in cancers associated with aromatic amine exposure. Glutathione S-transferase theta 1 (GSTT1), which is expressed in hematopoietic cells, is polymorphic and the lack of GSTT1 enzyme activity is due to an inherited deletion of the GSTT1 gene (null genotype). We have found that smokers with the GSTT1 null genotype have significantly higher levels of smoking-induced ethylene oxide-hemoglobin adducts and a higher frequency of smoking-induced mutations at the glycophorin A (GPA) locus in erythrocytes. Consistent with these observations that GSTT1 modulates exposure-related damage in blood cells, we find that the null genotype for GSTT1 is a significant genetic risk factor in myelodysplastic syndrome (MDS), a proliferative disease of the bone marrow that has been linked with chemical exposures. MDS patients were 4.3-fold more likely to carry the inherited GSTT1 null genotype ($p < 0.0001$).

Relevant Publications:

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The Statistical Modeling and Risk Assessment Group conducts and coordinates research into the development and use of biologically-based mechanistic models for characterizing and quantifying human health risks associated with exposure to environmental agents. This involves an active research program in computer-based mathematical modeling, ranging from efforts at the cellular and molecular levels to whole animals and focused on describing and evaluating chemical structures, biological response mechanisms and their perturbations by potentially hazardous environmental agents. This group's research activities cover four broadly-based areas: risk assessment, stochastic modeling of carcinogenesis, physiological/pharmacological/biochemical modeling, and predictive toxicology.

Relevant Publications:

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My research is directed toward understanding the interaction between genes and environmental exposures in human carcinogenesis. There are two main elements to this work, one directed at investigating the role of environmental exposure in critical target gene mutation and one directed at investigating the role of genetic susceptibility and environmental exposure in cancer risk.

The research on critical target genes addresses the hypothesis that different environmental exposures cause different patterns of mutation in genes that are important in carcinogenesis. My initial focus has been on mutational activation of oncogenes and deactivation of tumor suppressor genes. Most of my work has been on lung and bladder cancer, two tumors that have strong environmental determinants. In a recent study we showed that roughly one third of large and squamous cell lung tumors from uranium miners had an identical mutation in the tumor suppressor gene p53. This is one of only four known examples of an exposure-specific pattern of critical target gene mutation in human tumors. Such patterns can be used both to identify novel critical target genes and to suggest mutational mechanisms by which an environmental agent causes cancer. If specific carcinogens produce characteristic patterns of gene mutation in tumors, the detection of those patterns would be a powerful tool in studies of environmental risk and for use in prevention and early diagnosis.

My research on genetic susceptibility tests the hypothesis that commonly inherited allelic variants of selected candidate genes, in conjunction with environmental exposures, affect a person's risk of developing cancer. We are studying inherited polymorphisms in selected genes that have potential links to bladder cancer risk: genes involved in carcinogen metabolism, proto- oncogenes, tumor suppressor genes, and genes involved in DNA synthesis and repair. Working with genetically susceptible subgroups may allow us to identify the environmental exposures that cause disease and the true risks associated with exposure. It could also lead to public health programs for protecting susceptible populations, and for targeted screening of groups at higher risk of disease.

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Cell and Developmental Biology

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Studies in polypeptide hormone action.

Our laboratory has been interested in several aspects of signal transduction resulting from binding of polypeptide hormones to their surface receptors on cells. One major topic under study is the role of direct substrates for protein kinase C (PKC) in mediating the many cellular effects resulting from activation of this family of kinases by hormones and other agonists. We have been studying a small family of PKC substrates consisting of MARCKS and its smaller homologue, the MARCKS-like protein or MLP. Ongoing projects include structure-function studies of the protein and its mutant derivatives in two major systems, development of the mouse central nervous system, and early embryogenesis in *Xenopus laevis*. We are also studying gene promoter elements in these two species to determine which elements are important for the developmentally regulated, tissue-specific and cytokine-induced expression of these genes. We are investigating potential interactions of these proteins with a protease that specifically cleaves MARCKS, cathepsin B, and potential roles of these interactions in growth and metastasis of certain tumors, especially human breast cancer. Finally, we are investigating the possibility that mutations in the MARCKS and MLP genes are involved in human neural tube defects, particularly at the level of increasing a genetic predisposition to environmental causes of these defects.

A second major area of study in the laboratory began with the cloning of a gene that was rapidly and massively induced by insulin. The protein encoded by this gene, known as TTP, is the prototype of a novel class of CCCH zinc finger proteins; however, no function has yet been proven for any member of this class of proteins. We have shown in the past that TTP is rapidly induced, translocated from the nucleus to the cytosol, and phosphorylated on serine residues by insulin and by many other mitogens and growth factors. In addition, mice deficient in this protein develop a complex syndrome consisting of arthritis, wasting, dermatitis, and early death; more recent work has identified an excess of circulating tumor necrosis factor (TNF)(as the cause of most if not all aspects of the syndrome. We have shown that TNF(is over-produced by macrophages derived from these knockout mice, and that this is secondary to enhanced stability of TNF(mRNA in these cells. More

recently, we have found that TTP can bind to specific regions in this mRNA, and stimulate both the removal of the polyA tail of the mRNA and promote its rapid turnover. Current studies are attempting to elucidate the molecular details of this interaction; to identify other important mRNAs whose stability is regulated by TTP in normal physiology; to identify TTP binding proteins, which might modulate its activity; to knock-out the two known mammalian relatives of TTP in the hope of producing other informative phenotypes; and to investigate possible abnormalities in human autoimmune disease.

Relevant Publications:

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Many genes are expressed in male germ cells that: 1) encode unique proteins, 2) are regulated developmentally, 3) and are either transcribed only in male germ cells or produce mRNAs specific to these cells. The underlying hypothesis of our research is that such genes encode proteins with key structural or functional roles in the successive mitotic, meiotic, and post-meiotic phases of male germ cell development. Some of these proteins are germ cell-specific members of protein families, some are products of variant transcripts, and yet others are products of unique genes. In some cases, the gene encoding a protein isoform expressed in somatic cells is down-regulated, while a gene encoding a germ cell-specific isoform is up-regulated. The new protein presumably has properties that are advantageous to germ cells. However, the presence of unique proteins may also render male germ cells more susceptible than other cells to environmental chemicals.

The major aims of our studies are to determine if germ cell-specific proteins are responsible for the unique and essential events of germ cell development, resulting in the production of sperm that contain an intact haploid genome and are structurally and functionally competent to fertilize the egg. The strategy chosen has been to identify genes expressed in different phases of germ cell development, to define the roles of the proteins encoded by some of these genes and of genes characterized by collaborators, and to determine how selected genes are regulated developmentally. Regulation may occur directly through the intrinsic developmental program of germ cells, or through extrinsic endocrine or paracrine signals that indirectly influence gene expression.

Relevant Publications:

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Members of the nuclear receptor superfamily consist of ligand-dependent transcriptional factors that regulate a wide variety of physiological processes, including development, cell growth, apoptosis and differentiation. This superfamily includes the steroid hormone and retinoid receptors, and orphan receptors for which the ligand has yet to be discovered. Alterations in their receptor signaling pathways have been linked to several diseases, including cancer, diabetes and atherosclerosis. Development of agonists and antagonists offer tools to interfere in disease processes and may result in novel therapies. Three novel nuclear orphan receptors, retinoid-related orphan receptor γ(RORγ), TAK1 and RTR have been identified by our group. The research in the Cell Biology Group is focusing on the study of the biological functions of these receptors and their roles in disease. In addition, the mechanism of action by which these nuclear receptors regulate target gene expression is being investigated. The interaction of these receptors with hormone response elements, their transactivation activity and interaction with other nuclear proteins is being studied. To identify the physiological functions of ROR , a member of the nuclear receptor superfamily, mice deficient in RORγ function were generated by targeted disruption. RORγ$^{-/-}$ mice lack peripheral and mesenteric lymph nodes and Peyer's patches indicating that RORγ expression is indispensable for lymph node organogenesis. The thymus of RORγ$^{-/-}$ mice contains 75% fewer thymocytes than that of wt mice. Flow cytometric analysis showed a decrease in the CD4^{+}CD8^{+} subpopulation. TUNEL staining demonstrated a four-fold increase in apoptotic cells in the cortex of the thymus of RORγ$^{-/-}$ mice. This was supported by the observed increase in annexin V-positive cells. RORγ$^{-/-}$ thymocytes placed in culture exhibit a dramatic increase in the rate of "spontaneous" apoptosis. This increase is largely associated with CD4^{+}CD8^{+} thymocytes and may at least in part be related to the greatly reduced level of expression of the anti-apoptotic gene Bcl-X_L. These studies indicate that RORγ is essential for lymphoid organogenesis and plays an important regulatory role in thymopoiesis. Our findings support a model in which RORγ negatively controls apoptosis in thymocytes.

Relevant Publications:

Yan, Z-H., Medvedev, A., Hirose, T., and Jetten, A.M. Characterization of the response element and DNA-binding properties of the nuclear orphan receptor germ cell nuclear

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Environmental agents produce a variety of effects on the reproductive tract, some of which result in infertility and toxicity. In many cases such effects are direct and produced by agents having estrogenic hormonal activity, but with little structural resemblance to the natural ligand. Mechanistically, hormonal activity is believed to be mediated through an intracellular receptor protein, although the tissues and specific responses vary, a primary response is increased gene transcription. The receptor demonstrates specific stereochemistry for endogenous compounds but appears to interact less selectively with exogenous chemicals. In order to better understand such differences, studies are underway to determine a structural and chemical basis for the stimulation of estrogenic responses and the involvement of the estrogen receptor in this process. Two major tissue systems are investigated including the reproductive tract and bone. Reproductive tract tissue is a principal target site of estrogen action related to hormone responsiveness being investigated at the biochemical and molecular level. Bone tissue has been known to be susceptible and sensitive to estrogen withdrawal and treatment. Until recently the effect was believed to be indirect, however, the description of estrogen receptors in bone tissue indicates estrogen can act directly. Investigations are involved to identifying the estrogenic responses in bone cells and evaluate the different cellular mechanisms involved in their activation.

Research in the Receptor Biology Section involves three major interrelated approaches. First, the structural basis of ligand interactions with the estrogen receptor is investigated in order to more fully understand the importance of the ligand binding to nuclear estrogen receptor interactions and its role in stimulating estrogenic responses. Gene transfection studies are being used to examine the influence of different ligand structures and receptor forms on the regulation of exogenous hormonally responsive reporter gene constructs. Second, the biochemical and molecular properties of the estrogen receptor protein are analyzed to determine what processes are involved in its activation and role in mediating biological responses. Steroid hormone receptor protein modifications may function to modulate the activity and specificity of responsiveness as proposed for other signaling systems. Finally, the group is investigating the expression of uterine estrogen responses and evaluating the possible coupling of other signal transduction mechanisms such as growth factors and their involvement in the mechanism of uterine stimulation to aid in determining an overall understanding of estrogen hormonal tissue responses. Experimental approaches involve tissue culture;

transgenic animal models including insertional and knock-out transgenics; nucleic acid biochemistry and gene cloning; and protein biochemistry, purification, and characterization.

Relevant Publications:

Ignar-Trowbridge, D.M., Nelson, K.G., Bidwell, M.C., Curtis, S.W., Washburn, T.F., McLachlan, J.A., Korach, K.S. (1992) Coupling of dual signaling pathways: Epidermal growth factor action involves the estrogen receptor. Proc. Natl. Acad. Sci. USA 89:4658-4662.

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The overall goal of the Cell Biology Section is to develop an understanding of the mechanisms involved in cell injury and death. Particular attention has been devoted to the role of ionic and biochemical alterations which are involved in cell injury. We hypothesize that environmental stress initiates a cascade which, depending on the size and duration of the stress, as well as the cell type, can lead to either the development of cell protection, apoptosis or necrosis. The Cell Biology Section is elucidating the signaling pathways responsible for protective adaptation and apoptosis. To accomplish this goal two model ystems are studied: 1) signaling pathways involved in cardio-protection and, 2) signaling pathways involved in apoptosis. We find that stressing cardiac tissue activates a signaling cascade involving PI3-kinase, protein kinase C, which in turn activate NO synthase and the lipoxygenase and epoxygenase pathways of eicosanoid metabolism, leading to cardioprotection. We are currently investigating the interaction among these signaling pathways and their possible involvement in regulating sarcoplasmic reticulum (SR) calcium and cytosolic calcium as well as altering K and Ca channel activity. We have recently used a new NMR indicator to measure in situ SR ionized calcium. We report a basal SR ionized Ca2+ concentration of 1 mM. In another set of studies we are investigating mechanisms of cell injury using transgenic mice to study the role of specific gene products. We have also used transgenic mice to investigate the mechanisms responsible for gender related differences in susceptibility to injury.

Another area of investigation involves understanding signaling pathways involved in apoptosis. Evidence is emerging that a decrease in endoplasmic reticulum (ER) calcium is involved in signaling apoptosis. Our current research is directed to define the mechanism(s) responsible for the decrease in ER Ca2+ and apoptosis. We find that low serum induced apoptosis leads to a decrease in ER calcium secondary to reduced calcium entry across the plasma membrane. We further find that perturbing vesicle trafficking by treatment with bafilomycin leads to apoptosis. We have also begun to investigate the mechanism by which a decrease in ER calcium leads to apoptosis and we find that decreased ER calcium leads to generation of ceramide. We also find that altered calcium homeostasis leads to altered levels of NF-kB , which modulate apoptosis.

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Cytochrome P450 enzymes and sulfotransferases (STs) represent two large groups of enzymes which play key roles in the First Phase and the Second Phase metabolism of xenobiotics and steroid, respectively. Characteristically, these enzymes display remarkably diverse substrate specificities to metabolize numerous endogenous substrates including steroids, lipids and neurotransmitters, as well as exogenous chemicals such as drugs, environmental pollutants and procarcinogens. In addition, P450s and STs are induced by various xenobiotic chemicals as well as endocrine signals such as growth hormone. As a cellular defense mechanism against the toxicity and carcinogenicity, the induction of and metabolism by P450s and Sts usually lead to increased detoxification and elimination of xenobiotics. Paradoxically, however, they can often result in the bioactivation of toxic and carcinogenic xenobiotics. Thus, understanding the molecular mechanisms of the substrate specificity and induction process is critical to delineate the roles of the P450s and STs in human susceptibility to environmental toxins and carcinogens. Site-directed mutagenesis studies have identified that few key amino acid residues regulate the substrate specificity of the P450s. The high resolution diffraction data are collected from crystals of STs. A mouse primary hepatocyte system has been established and used to determine a phenobarbital-responsive enhancer module (PBREM) of a P450 gene. A PRBM-binding nuclear protein is purified and its cDNA is being cloned. The sex-specific P450 genes are found to exhibit the sexually dimorphic demethylations. Various nuclear and cytoplasmic proteins have been investigated in respect to their capacities to transduce growth hormone signals and to recognize demethylated P450 promoters.

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Research in this group is concerned with trying to understand, at the cellular and molecular level, how cells regulate calcium and how hormones and neuro-transmitters utilize calcium as a cellular signal. An early event following the activation of receptors in this class is the hydrolysis of a membrane phospholipid, phosphatidylinositol 4,5-bisphosphate, to yield two putative second messenger molecules, diacylglycerol and inositol 1,4,5-trisphosphate. Diacylglycerol activates protein kinase C, and inositol 1,4,5-trisphosphate releases calcium from an intracellular organelle. In addition to the release of intracellular calcium, receptor activation also leads to an increased entry of calcium into the cell across the plasma membrane. This calcium entry is activated by a signal generated by depletion of intracellular calcium stores. In single cells, the pattern of calcium signalling often takes the form of discrete calcium spikes or oscillations which investigate the mechanisms underlying the phenomena of intracellular calcium release and entry, as well as the mechanisms by which calcium oscillations arise, by combining the techniques of cellular microinjection, whole cell and single channel patch-clamp, single cell calcium analysis, calcium imaging, and molecular biology.

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Research Summary

Our research interests can be divided into three main areas: (a) cytochromes P450 and the bioactivation of arachidonic acid; (b) cyclooxygenase-derived eicosanoids and lung function; and (c) asthma: exposure assessment and prevention.

Cytochromes P450 and the bioactivation of arachidonic acid. Our work in this area has been driven by two hypotheses: (1) cytochromes P450 metabolize arachidonic acid to eicosanoids that play critical roles in modulating fundamental biological processes; and (2) aberrant P450 expression/activity due to environmental and/or genetic factors leads to altered production of bioactive eicosanoids and results in cell/organ dysfunction and disease. Our group's recently developed CYP2J2 transgenic mice have improved post-ischemic cardiac function and represent the first *in vivo* animal model to evaluate P450 functions in heart. We demonstrated that CYP2J2-derived eicosanoids decreased cytokine-induced endothelial adhesion molecule expression by a mechanism that involves inhibition of the transcription factor NF-κB. We also showed that exposure of endothelial cells to hypoxia-reoxygenation decreased CYP2J2 expression, that maintenance of CYP2J2 protein levels attenuated hypoxia-reoxygenation-induced cell death, and that CYP2J2 products affected endothelial capacitative calcium entry.

P450-derived eicosanoids have been extensively studied in the kidney and have been shown to contribute substantially to integrated renal function. We reported the cloning of CYP2J5, a new mouse P450 that is primarily expressed in the kidney, active in the metabolism of arachidonic acid to EETs, and localized to proximal tubules and collecting ducts, sites where EETs are known to affect renal fluid/electrolyte transport and mediate the actions of several hormones.

In addition to the CYP2J work, we have cloned and characterized CYP2C8, the major human liver P450 arachidonic acid epoxygenase, and characterized several mouse CYP2C isoforms. Recently, our group has identified a new P450 subfamily, the CYP2Ns, which are phylogenetically related to the CYP2Js,

abundant in extrahepatic tissues and active in the metabolism of arachidonic acid to EETs .

Cyclooxygenase (COX)-Derived Eicosanoids and Lung Function. Our work in this area is based on the hypothesis that COX-derived eicosanoids are important modulators of the lung immune response to environmental agents and that COX deficient mice provide a novel model system to study basic immunologic mechanisms operative during the development of inflammatory lung disease. Our proposed studies on the role COX-derived eicosanoids in modulating the pulmonary immune response to environmental agents and in the pathogenesis of asthma will provide unique opportunities for both mechanistic and translational research.

Asthma: Exposure Assessment and Primary Prevention. Our asthma research program involves both exposure assessment and primary prevention components, and focuses on the relationship between exposure to common indoor allergens and asthma prevalence / morbidity. The National Allergen Survey, a descriptive study of allergen types and levels in floor and bedding dust in the nation's housing, is the first study to provide estimates of allergen exposure in the entire U.S. population. Preliminary results from analysis of dust mite allergens suggest that approximately 23% of U.S. homes have beds that contain >10μg / gram dust mite allergen (a level previously associated with symptomatic asthma) and approximately 46% of U.S. homes have beds that contain >2μg / gram dust mite allergen (a level previously associated with mite allergen sensitization).

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Steroid hormones regulate tissue-specific gene expression in animals via receptor dependent intracellular signal transduction pathways. These receptors, when activated by the appropriate ligands, both activate and repress the transcription of subsets of genes in target cells which results in altered gene expression and altered function. We are particularly interested in glucocorticoid receptors and their actions because they reflect the primary response to environmental stress. Current research projects are examining the following aspects of glucocorticoid hormone action: (1) mutual interference of signaling between the glucocorticoid receptor and NF-KB; (2) the role of receptor phosphorylation in signal transduction; (3) the regulation of glucocorticoid receptor gene expression; (4) the involvement of the beta form glucocorticoid receptor in the generation of steroid resistance.

A second major interest of the laboratory focuses on evaluating the mechanisms involved in the regulation of apoptosis in normal and neoplastic cells. Research is aimed at the identification and cloning of genes that are responsible for the initiation and execution of apoptosis. Current projects include: (1) identification of nucleases that cleave chromatin during apoptosis; (2) evaluation of the role of ribosomal RNA degradation in apoptosis; (3) cloning of inhibitors of apoptosis; (4) the role of cell volume regulation and ion fluxes in the activation of apoptosis; (5) apoptosis in yeast; (6) evaluation of the role of NFB in apoptosis.

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Dr. Mishina focuses on the characterization of signal transduction pathways important in mammalian development. His research focuses on the role of bone morphogenic proteins (BMPs) and BMP receptors in murine development. Dr. Mishinaís research has linked BMP2/4 signaling to a critical inductive role in mesoderm formation during gastrulation. Currently, his research focuses on using BMP2/4 knockout mice and the BMP-receptor knockout mice to characterize BMP signaling pathways and associated downstream targets and gene responses. These studies are being extended to produce tissue specific BMP-receptor knockouts in chondrocytes and bone tissue. These transgenic and knockout mouse lines will be used to do comparative studies on Mullerian-inhibiting substance and BMP signaling pathways to determine their respective roles in mammalian development and to evaluate the activity of environmental chemicals in perturbing BMP signaling that results in developmental toxicity and teratogenesis.

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The focus of our group is to understand the role of adaptor proteins in the integration of signal transduction cascades. In particular, we are interested in proteins that modulate the function of receptor tyrosine kinases (RTKs). A major target of numerous RTKs is the Shc family of adaptor proteins. This family of proteins lacks an enzymatic domain but consists entirely of modular protein:protein recognition motifs incuding Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains. We have identified a novel Shc protein, ShcC, which is highly restricted in its pattern of expression to the central nervous system. Expression of ShcC is induced in differentiating neurons as well as in regions of the developing mouse brain undergoing differentiation. Interestingly, ShcC is expressed in neuroblastoma tumors but is absent in other nervous system tumors suggesting a potential role for ShcC in carcinogenesis. In support of this notion, loss of ShcC expression in neuroblastoma tumors is associated with an alteration of the phenotype of these cells. We are currently examining the role of ShcC in neural development and signaling and are particularly interested in determining the importance of ShcC in neuroblastoma tumors. Although the members of the Shc family are well conserved in sequence, current evidence from our group, as well as others,suggests distinct functions of the various Shc family members and as such we are interested in understanding these activities.

In addition, our group is studying a novel adaptor protein, intersectin, which like ShcC, consists of protein:protein interaction modules. Intersectin is composed of two amino-terminal Eps-15 homology (EH) domains, a central coiled-coil domain and 5 Src homology 3 (SH3) domains. Intersectin is an important component of the endocytic machinery where it is thought to regulate the internalization of a number of membrane associated proteins. However, my laboratory has recently uncovered a novel function for intersectin in the regulation of signal transduction cascades. We have shown that intersectin is capable of activating signal transduction pathways to regulate cell growth and differentiation. Recent data from a number of groups suggests that endocytosis and mitogenesis may be linked in some manner. Our findings solidify this link between the endocytic machinery and signal regulation. Interestingly, there is a larger isoform of intersectin that is restricted in expression to the brain. The sequence of this larger version of intersectin suggests that it will regulate the Rho family of small GTPases which are part of the larger Ras superfamily. In addition, Intersectin has been mapped to human chromosome 21 in the Down Syndrome region. Together, these

finding suggests that intersectin overexpression in Down Syndrome may contribute to sequelae associated with Down Syndrome. We are currently examining this possibility through the use of mouse models. Further work by our group will help to elucidate the mechanism of intersectin function.

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Transcription in Breast Cancer Cells

Summary – Steroid hormones, such as estrogen and progesterone, play an important role in the development and treatment of breast cancer. In my laboratory, the scientists have undertaken detailed analysis of the mechanism of action of the steroid receptors and clinically important steroid receptor antagonists that are used to block their action. Recently, we have expanded our research focus to encompass a mechanistic examination of environmental agents that act as endocrine disrupters in the reproductive process and in mammary cells.

Steroid hormones act via a group of high affinity receptors that regulate cellular growth and development by binding to the promoters of hormone inducible genes. In higher animals, including humans, DNA is organized with nuclear proteins to form chromatin which plays an important role in controlling gene expression. A thorough understanding of steroid hormone action has to accommodate the fact that their receptors function in concert with other transcription factors within chromatin.

Current Research Projects – The mouse mammary tumour virus (MMTV) promoter provides a excellent model for studies of hormone-regulated transcriptional control in the context of chromatin. The research in my laboratory is designed to define the mechanisms responsible for steroid regulation of transcription in vivo. In recent experiments we have shown that the MMTV promoter assembled in chromatin was refractory to progesterone stimulation, but was highly responsive to glucocorticoid. In contrast, both steroids activate transcription from the same reporter cassette when transiently introduced into cells. Results with human breast cancer cells that contain only the progesterone receptor demonstrate that the chromatin adopts an "open" structure that allows transcription factors to bind in the absence of hormone. This novel finding has provided a new level of understanding of how steroid hormones regulate gene transcription and how chromatin influences this fundamental biological process. We have taken advantage of our novel human and mouse breast cancer cell lines to describe the mode of action of these hormone antagonists. Our studies demonstrate that antagonists can be divided into at least two functional groups: antagonists that permit receptor interactions with genetic material and those that do

not allow it. It is expected that further studies of these important events should improve our knowledge of hormone action and reveal novel ways of controlling steroid-dependent disease processes. Studies with antagonists provide a framework for our expanded investigations of environmentally important endocrine disrupters.

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Cancer Biology

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Cell adhesion and migration contribute to normal processes such as cellular differentiation, embryonic development, and wound healing as well as to the progression of diseases and pathological conditions that can result from either acute or chronic exposure to environmental toxicants, such as cancer and inflammatory responses. Such cell adhesive processes result from the interactions of extracellular glycoproteins such as fibronectins, laminins, and collagens with specific receptors, the best characterized of which are the integrins. Integrins are all non-covalent, heterodimeric complexes consisting of an alpha subunit and a beta subunit. The major interest of this research group is to characterize the molecular mechanisms of integrin-mediated adhesion processes, integrin activation, and the resulting downstream processes induced by adhesive proteins such as fibronectin important for the control of proliferation, adhesion, migration, and invasion of cells, especially focusing on human tumor cells. The primary approaches use monoclonal antibodies, protein and peptide biochemistry, physical biochemistry, and cell and molecular biology to characterize the structure and function of fibronectin and its integrin receptors. Integrins can exist in both an active or inactive state. Optimal ligand binding requires that an integrin is in the activated state. Integrin ligands, divalent cations (especially manganese ion), and certain anti-betal monoclonal antibodies can all activate integrins directly. Integrin activators were initially identified by their ability to promote or increase cell-substrate adhesion, but they can also modulate other processes such as cell-cell adhesion and signaling pathways. The biochemical and biological consequences of integrin activation are currently being characterized. Integrin-mediated cell-cell adhesion appears to be the result of signaling processes can be very complex affecting multiple systems including cytoskeletal proteins and second messenger signaling pathways, leading to biochemical changes such as protein phosphorylation and intracellular pH, and eventually modulation of cell proliferation.

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Neoplastic development of cancers is a multistep process requiring multiple genetic changes. Significant advances have been made in the elucidation of the genes involved in genetic predisposition to cancer but less is known about the genes involved in the later stages of malignant progression. Identification of the target genes for different steps in the cancer process is important in understanding the environmental causes of cancer as well as the endogenous causes of cancer, which include spontaneous mutations, aging, and hormones. The role of aging in cancer is studied by cloning and characterizing genes involved in cellular aging. Unlike tumor cells, normal cells have a finite lifespan and enter a state of irreversible growth arrest, termed cellular senescence, at the end of their lifespan. We have shown that cellular senescence is genetically controlled and that multiple senescence genes are altered in immortal cancer cells. Only a few senescence genes have been identified and efforts to clone new genes are actively being pursued. Studies of the regulation of these genes by environmental factors may elucidate the causes of aging and cancer.

Another area of active investigation is the mechanisms of metastatic progression. The malignant phenotype of a cancer cell is under both positive and negative controls but little is known about the genes that control metastasis. We have recently cloned a novel metastasis suppressor gene, KAI1, which may be important in prostate, breast, lung, and possibly other cancers. Further studies of this and related genes may yield important new insights into cancer diagnosis and treatment. In addition, molecular markers for the later stages of cancer progression may help define the environmental factors that influence malignant development.

Hormones are major factors in human cancers and the Cancer and Aging Section is actively involved in studying multiple aspects of hormonal carcinogenesis, including molecular alterations of hormonally associated cancers (breast, prostate, and endometrial), mechanisms of estrogen-induced chromosomal changes, and the role of BRCA1 in regulating growth and tumorigenicity of breast cancer.

Oxidative stress is a major form of endogenous and exogenous damage to cells. The role of oxidative stress in cell senescence and cell death (apoptosis) is under study. Interestingly, the same genes (e.g., p53, Rb) are involved in cell senescence and cell death. A better understanding of the molecular mechanism of signal transduction

leading to cell senescence or cell death through divergent pathways is required to understand cellular responses to environmental stresses, particularly oxidative damage. As cells progress to cancer, their responses to apoptotic signals change. Interestingly, cancer cells die at a higher rate than normal cells, suggesting that environmental modulators of apoptosis can influence the rate of tumor growth. One example of this is dietary restriction of animals, which reduces cancer progression by stimulating apoptosis of precancerous cells. We have shown that this is in part due to modulation by dietary restriction of circulating IGF1 levels, which blocks apoptosis of cancer cells. Further studies on the genetic controls of apoptosis may help elucidate the role of diet and other environmental factors in cancer.

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Metastasis is the principal cause of morbidity and mortality in cancer patients. We are exploring characteristics of metastatic cells, such as adhesion and motility, that are critical for the successful spread of cancer. Current work focuses on the role of integrin complexes in the adhesion of human breast carcinoma cells to basement membrane proteins. We have shown that this adhesion is stimulated by cis-polyunsaturated fatty acids, such as arachidonic acid, and that the stimulation is dependent on protein kinase C and tyrosine kinase activities. We have begun to define the molecules involved in this adhesion and to elucidate specific signal transduction pathways that regulate the stimulation of adhesion by physiological agents. A second area of emphasis is the role of protein glycosylation in tumor cell biology. We have previously shown that an inhibitor of protein glycosylation, swainsonine, reduces tumor growth and metastasis in a murine model system, enhances the host immune response, and stimulates bone marrow progenitor cells. We are pursuing the pathways by which this compound affects cells, such as adherence to basement membrane proteins, cytokine production, protein kinase C activity, and Ca++ transport. The ability to alter the characteristics of various components of the hematopoietic system could have multiple applications in cancer therapy.

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This group conducts research in animals and in *in vitro* systems to understand the role of specific genetic and epigenetic events in the induction and development of skin cancers. Efforts are also directed toward the evaluation and use of transgenic models in carcinogenesis bioassays. A major goal of these efforts is to improve our understanding and ability to identify and classify potential carcinogens.

The major research objectives of the Cancer Biology Group are to: 1) understand the mechanisms underlying the very earliest events in the neoplastic cascade, and 2) understand the mechanisms of environmental carcinogenesis, goals shared by a number of laboratories around the world. Our studies, however, have unique aspects that are related to, or are derivatives of, the problem of identifying carcinogens. There has been remarkable progress over the past decade in identifying specific mutated genes that occur in human cancers such as retinoblastoma, Wilm's tumor, and adenocarcinoma of the colon. These accomplishments have strengthened the concept that multiple, time-dependent genetic changes are involved in the development of malignancies. However, genetic changes are difficult to resolve either into causal versus consequential events, or into effects that are the result of increasing genomic instability. Thus, despite advances in molecular methodologies that allow human cancers to be studied directly, researchers must still depend upon animal models to study the processes by which neoplasias arise.

The murine multistage epidermal carcinogenesis model has provided a unique opportunity to understand the genetic and morphological changes that accompany the transition of an epithelial cell from normal to neoplastic. Skin models in particular have played a significant role in establishing the concepts of initiation, promotion, and progression in tumor development, and - since the majority of human cancers are epithelial in origin - these models are relevant to understanding human carcinogenesis. Studies on the process of malignant conversion from a benign cutaneous papilloma to a squamous cell carcinoma have provided not only an important system for the identification of critical genetic changes driving the neoplastic process in epithelial cells, but also insights into the regulation of epidermal growth and differentiation. In addition, mouse skin models have identified chemicals with the capacity to promote tumor development.

In the past decade, technical advances have given us the capacity to genetically manipulate the mammalian genome. A product of this technology was the development of transgenic mice, which have altered expression of specific genes, often targeted to specific tissue types. Transgenic mouse models are particularly important in defining cellular and molecular processes involved in chemical carcinogenesis, since they have the inherent capability of allowing assessment of tissue distribution and chemical metabolism, both of which are deficiencies of *in vitro* systems. The research program of the Cancer Biology Group has extensively utilized transgenic mouse models, with a particular focus on the v-Ha-*ras* Tg.AC mouse skin carcinogenesis model. Mutation of the endogenous c-Ha-*ras* oncogene has been frequently observed in both mouse and *in vitro* models, and is considered to be an important early event governing neoplastic transformation in the mouse skin model. Although evidence supports a role for the *ras* oncogene in papilloma induction and malignant progression, its role is complex and involves the action of other genes. The transgenic Tg.AC mouse was produced by the introduction of an activated v-Ha-*ras* oncogene via pronuclear injection into FVB/N mice (Leder, et al. 1990), and has the unique property of being genetically initiated. We have recently made some significant observations related to papilloma induction in these mice, as well as the use of the Tg.AC and other models as bioassays for carcinogens. This work is described in the manuscripts listed below.

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Biophysics/Structural Biology

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The Nucleic Acid Enzymology Section conducts physical and biochemical studies of mammalian DNA polymerases and in particular, studies gap-filling DNA synthesis during DNA repair. To define the biological role of DNA polymerase ß, the group and collaborators recently constructed DNA polymerase "knock-out" cell lines from a transgenic mouse model. These cell lines are devoid of all DNA polymerase ß mRNA and protein, and cell extracts lack base excision repair capacity, thus establishing the requirement of this particular DNA polymerase for the short gap-filling DNA synthesis required in uracil-initiated base excision repair.

The group and collaborators have reported many crystal structures of complexes of rat and human DNA polymerases with the two substrates (DNA and dNTP), and the group and collaborators have solved the NMR structure of the enzyme's 8 kDa domain. This work has improved our understanding of the fundamental mechanism of DNA synthesis and of the phenomenon of templating. The group also studies gene xpression control for DNA polymerase ß, since base excision repair capacity is a tightly regulated process in mammalian cells.

Finally, the research program also includes studies of structure-function relationships of the HIV-1 Reverse Transcriptase. The group and collaborators have conducted extensive kinetic studies of RT-nucleic acid interactions. This work provides a framework for drug design and for biochemical analysis of the relationship between the structure of the reverse transcriptase and its functions. The recombinant expression system for this enzyme developed by the group and studies of frameshift mutagenesis in collaboration with Dr. Kunkel and colleagues have facilitated understanding of this important reverse transcriptase.

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The Macromolecular Structure Group uses the tools of structural biology, primarily x-ray crystallography, to study the overlapping areas of embryonic development, cell signaling, and RNA-protein interactions. Many macromolecules involved in the processes of development, signaling, and RNA transactions are regulators of cell growth and differentiation. Thus fundamental knowledge about the structure and function of these macromolecules will contribute to our understanding of diseases such as cancer where environmental influences have resulted in aberrant growth and signaling and will also provide a structural framework for the design of therapeutic compounds that induce or inhibit specific signaling pathways.

The current projects in the Macromolecular Structure Group include structural studies of proteins involved in post-transcriptional gene regulation and a model system for ribonucleoprotein machines. The first project focuses on proteins that are involved in regulating messenger RNA (mRNA) stability by binding to adenosine-uridine (AU)-rich elements (AREs) in the 3' untranslated regions of some mRNAs. These AREs have been shown to confer instability on the transcripts that contain them and are important players in regulating gene expression. We have recently determined the crystal structure of an ARE-binding fragment of an Hu protein bound to minimal fragments of the cfos and TNF alpha AREs. These structures identified residues important for RNA recognition and suggest a consensus RNA recognition sequence for Hu proteins. Further biochemical and functional assays will allow us to refine this consensus sequence.

The second project focuses on a one protein-one RNA ribonucleoprotein catalyst to provide insight into complex, multicomponent ribonucleoprotein machines such as the ribosome or spliceosome. The system is the fifth intron of yeast mitochondrial cytochrome b pre-mRNA (bI5) and the protein CBP2 (cytochrome b pre-mRNA processing protein 2). The bI5 intron is a group I self-splicing intron. It contains the active site for the splicing reaction, but the protein co-factor, CBP2, assists in the reaction by holding the RNA in its active conformation. Determining the crystal structure of this simpler model system will allow the correlation of structure and function and should produce some general principles that can be applied to more complex protein-RNA systems involved in chromosome maintenance, mRNA splicing, and protein synthesis.

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The NMR group uses magnetic resonance spectroscopy to study at the molecular level the structural and metabolic perturbations which can result from exposure to agents of environmental concern. These studies include structural characterizations of adducts which form between chemicals of environmental or pharmacological interest, and macromolecular targets or model systems. Recent studies have involved adducts formed from carbon-13 labeled aspirin and haloacetic acids, with the proteins ubiquitin and hemoglobin, as well as the labile boronate adducts formed with boric and boronic acids. The group is also interested in the structure of E. coli DNA polymerase III, in order to better understand how structural and chemical factors influence replication fidelity. Finally, studies of AIDS related proteins such as HIV protease are also in progress.

NMR methods are developed in concert with the programmatic work outlined above. Recent efforts have focused on developing dynamic shift multiplet pertrubations as a tool for evaluating molecular dynamics, extension of the transferred NOE experiment, and the development of NMR methods for the detection of radical protein adducts. Methods involving fluorine-19 labeling for NMR studies of larger systems are also under evaluation.

In addition to structural NMR work, studies of intracellular ions, and the development of methods to determine ion levels in cells are also done by the group. Recent work has focused on intracellular magnesium and its measurement.

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Dr. Ronald P. Mason is the workgroup leader of the Free Radical Metabolites orkgroup, which uses electron spin resonance to detect and identify free radical metabolites of toxic chemicals and drugs. The chemical reactions of free radical metabolites have biochemical and toxicological consequences that cause cellular damage and death. The group has pioneered the application of the electron spin resonance spin trapping technique to biochemical, pharmacological and toxicological problems with particular emphasis on the use of spin traps in vivo. The in vivo experiments are critical because, unless free radical metabolites can be demonstrated with a whole animal model, there will always be some question as to their actual existence in biology. With the aid of this technique the group has demonstrated the formation of free radicals from rancid unsaturated fatty acids, established the role of hydroxyl radicals in iron and copper toxicity, and implicated the involvement of an ethanol-derived free radical in alcohol-induced cirrhosis of the liver.

Currently the Free Radical Metabolite Workgroup is active in four areas of research. 1) The mechanism of free radical generation by the reaction of hemoproteins with hydroperoxides. The central unanswered question in these reactions is whether the alkoxyl radical or the peroxyl radical is the true product of reaction. We have been able to demonstrate that in every case examined thus far (i.e., cytochrome c, hematin, chloroperoxidase, and cytochrome P-450) that the alkoxyl radical is the species initially produced and that all other detected free radical are the result of the free radical chemistry of this species. 2) The detection, identification, and reactivity of myoglobin-derived radicals. The structures of the reactive free radicals formed by the reaction of metmyoglobin with hydrogen peroxide have been unknown since their discovery

nearly forty years ago. With the use of isotopic labeling, spin trapping, and molecular biology the oxygen-reactive free radical has been identified as tryptophan. 3) In vivo detection of metal-mediated free radical formation. Many investigations of acute metal toxicity have detected the in vivo formation of either the hydroxyl radical or alkyl radical products of lipid peroxidation. Recently, we successfully detected hydroxyl radical generation in rats with chronic dietary iron supplementation in the absence of liver toxicity and with only modest serum ferritin increases of the magnitude thought to lead to a greater risk of myocardial infarction in man. 4) The role of nitric oxide in the metabolism of toxic chemicals and drugs. In vivo nitric oxide production and its role in the synergistic carbon tetrachloride/endotoxin toxicity have been investigated. The in vivo metabolism of hydroxyurea to nitric oxide has been discovered.

Relevant Publications:

Barr, D.P., Martin, M.V., Guengerich, F.P., and Mason, R.P.: Reaction of cytochrome P450 with cumene hydroperoxide: ESR spin trapping evidence for the homolytic scission of the peroxide O-O bond by ferric cytochrome P450 1A2. Chem. Res. Toxicol. 9: 318-325, 1996.

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Iimuro, Y., Bradford, B.U., Gao, W., Kadiiska, M., Mason, R.P., Stefanovic, B., Brenner, D.A., and Thurman, R.G.: Detection of -hydroxyethyl free radical adducts in the pancreas after chronic exposure to alcohol in the rat. Mol. Pharmacol. 50: 656-661, 1996.

Jiang, J.J., Liu, K.J., Jordan, S.J., Swartz, H.M., and Mason, R.P.: Detection of free radical metabolite formation using in vivo EPR spectroscopy: Evidence of rat hemoglobin thiyl radical formation following phenylhydrazine administration. Arch. Biochem. Biophys. 330: 266-290, 1996.

DeGray, J.A., Gunther, M.R., Tschirret-Guth, R., Ortiz de Montellano, P.R., and Mason, R.P.: Peroxidation of a specific tryptophan of metmyoglobin by hydrogen peroxide. J. Biol. Chem. 272: 2359-2362, 1997.