LABORATORY OF CANCER PREVENTION: Nancy H. Colburn, Ph.D., Chief

Scientific Overview: The Laboratory of Cancer Prevention (LCP) investigates the molecular basis of cellular processes that, when perturbed, can lead to cancer induction and progression. Discovery and characterization of molecular targets for cancer prevention and intervention is a common area of interest among the seven principal investigators in the LCP. Mouse models engineered for resistance or sensitivity to carcinogenesis as well as non-engineered models represent a second unifying theme. LCP PIs study oncogenic transcription factors AP-1 and NFκB, oncogenic translation factor eIF4F, molecular chaperones, oncogenic protein kinases, inflammation associated genes such as COX-2 and HIF-1, selenoproteins such as chemopreventive antioxidant enzymes, and epigenetic processes such as DNA methylation that can silence tumor suppressor genes. Several of these molecular targets are being investigated to achieve the basic understanding of molecular interactions and mechanisms needed before subjecting them to the test as targets for cancer prevention or cancer intervention. Others have been validated in mouse and human models as targets for prevention; i.e. "hitting the target" is efficacious in preventing tumorigenesis or tumor progression. LCP PIs use mouse models to study gene function in normal processes or in tumor induction or prevention. LCP PIs conduct clinical prevention trials using nutritional or pharmaceutical interventions and make human samples available for analysis by LCP colleagues. LCP PIs collaborate with the NCI Division of Cancer Prevention, with the NCI Mouse Models of Human Cancer Consortium, with CCR's Molecular Targets Development Program, and with the NCI Developmental Therapeutics Program.

LCP investigators have made a number of paradigm-changing discoveries, reflected in the scientific community's high citation rate of LCP publications: LCP PIs can claim 43 career high-impact articles (i.e., greater than 90 citations). Since 2000, LCP investigators have generated more than 160 publications, of which twelve were high-impact (i.e., greater than 30 citations). Among the highlights of LCP investigators' accomplishments is Nancy Colburn's discovery that transcription factors AP-1 and NFκB drive oncogenesis while translation inhibitor Pdcd4 acts as a suppressor, thus identifying particular transcription factors and translation factors as promising targets for cancer prevention. Dolph Hatfield, the discoverer of selenocysteine tRNA, has developed novel mouse models to discern the role of selenium and selenoproteins in health and disease prevention. Stress related selenoproteins appear to be important in the mechanism by which selenium confers protection against tumorigenesis. Sandra Ruscetti has demonstrated that a leukemia virus uses specific kinases to produce growth factor independence and cell survival. Her studies implicate Jun-N-terminal kinase as a promising target for intervention. Kathrin Muegge discovered that SNF2 family member Lsh is a guardian of normal heterochromatin structure that controls genomic methylation. Lsh-modulated gene silencing may be important in inactivating certain tumor suppressor genes. Chou-Chi Li provided the first biochemical evidence that the chaperone p97/VCP targets ubiquitinated proteins. She established that VCP binds to poly-ubiquitinated proteins and targets these proteins to the proteasome for degradation. Elaine Lanza, the first to measure levels of fiber in the US diet, demonstrated that motivated, free-living individuals, given appropriate support, can make and sustain major dietary changes over a 4-year period. Moreover, the most compliant quartile in the Polyp Prevention Trial showed reduced risk of advanced adenoma recurrence. Iqbal Ali has made important contributions to the identification of response classifiers for the chemopreventive drug celecoxib. Douglas Ferris has contributed significantly to our understanding of the cell cycle associated enzyme Polo-like Kinase (Plk). George Beck discovered the signaling pathway activated by inorganic phosphate and showed that reducing phosphate levels reduces cancer risk in a mouse model. In collaboration with Mass Spectrometry experts Tim Veenstra and Tom Conrads at SAIC in Frederick, he advanced the science of systems analysis.

New initiatives and collaborations: Among the new initiatives and collaborations that involve LCP Investigators are

- Drs. Colburn, Lanza, and Young Kim, Division of Cancer Prevention (DCP) to develop and use mouse models that provide imageable readout of proinflammatory gene stimulation and its modulation by nutritional interventions.

- Drs. Hatfield and Cindy Davis (DCP) to ascertain using mouse models whether the chemopreventive activity of selenium can be attributed to selenoproteins or to small molecule forms of selenium.

- Dr. Ali with multiple extramural investigators to determine predictors of risk and therapeutic response especially related to COX-2 as a target.

- Dr. Lanza with multiple extramural investigators, particularly at Pennsylvania State and Michigan State Universities to ascertain the efficacy of high legume diets in polyp prevention.

- Drs. Colburn and Lanza with the CCR's new Inflammation and Cancer Program. Drs. Colburn and Lanza, with Drs. Scott Durum and Curtis Harris will co-organize a one-day retreat on Inflammation and Colon Cancer, with emphasis on targeting pro-inflammatory gene activation for cancer prevention.

- Dr. Colburn with Drs. Chand Khanna and Lee Helman of the Pediatric Oncology Branch, CCR to identify translationally regulated targets important for invasion and metastasis and to develop a drug discovery tool.

- Dr. Hatfield with Dr. Jeff Green, LCRC, CCR, to determine the possible efficacy of selenium intervention in a mouse model of breast and prostate cancer.

- Dr. Ruscetti with Dr. Michael Lerman, LIB, CCR, to determine whether a short form of the human protein kinase RON is oncogenic alone or in conjunction with retroviral envelope proteins and with Dr. Mary Carrington, LGD, CCR, to determine if polymorphisms in the putative promoter of short form RON correlate with resistance to cancer.

- Dr. Li with Dr. Virginia Kimonis (Harvard University) to study the pathological involvement of VCP mutations in a dominant fatal disease IBMPFD (Inclusion Body Myopathy associated with Paget disease of bone and Frontotemporal Dementia).

- Dr. Li with Dr. Toshi Mori (MTDP) to screen for the activators and inhibitors of VCP a molecule that may be developed into a therapeutic target for cancers and neurodegenerative diseases.

Administrative Overview: The Laboratory of Cancer Prevention was formed in November 2003, initially consisting of Drs. Colburn, Hatfield, Ruscetti, Li, Ferris and NCI Scholar Beck. During 2004 we were joined by Drs. Ali, Lanza and Muegge. In the current cycle Drs. Colburn, Hatfield, Ruscetti, Lanza and Ali will be reviewed. Dr. Muegge received a positive review in 2004. Dr. Li encountered serious illness in 2004-2005 but is happy to be back in the laboratory now. The intellectual antecedent to the LCP was the NCI Cancer Prevention Faculty formed in 2001 and the administrative antecedent was the Basic Research Laboratory formed in 1996. Dr. Colburn has

served the Cancer Prevention Faculty (CPF) as Chair since its inception. Dr. Lanza serves on the CPF Steering Committee and Drs. Lanza, Hatfield, Ali, Ruscetti, Beck, Muegge, and Li have contributed to CPF events. Among these Prevention Faculty events have been Retreats in 2001, 2003, and 2005 that were successful in stimulating interdisciplinary collaborations to translate cancer prevention research. Most of us were former members of CCR's Basic Research Laboratory. The fall of 2005 brings the departure of Dr. Ferris to college teaching and Dr. Beck to Emory University School of Medicine as tenure track Assistant Professor. As a sequel to his success as an NCI Scholar at the NCI, Dr. Beck takes a two-year K22 grant with him to Emory. Our laboratories and offices are in Buildings 567, 576, and 469 on the NCI Frederick campus, in Executive Plaza (EPN), and in Building 37 on the NCI Bethesda campus. To facilitate collaborations Dr. Lanza spends four days a week at EPN and one day a week working at NCI Frederick. We meet monthly in Frederick for presentations by LCP postdoctoral fellows and PIs, which often evoke lively discussions. A twice a month journal club features articles relevant to multiple Sections. To bring outside speakers to Frederick, an LCP seminar series is being established.

LCP Principal Investigators share a number of mouse model and DNA reagents with the scientific community and two (Drs. Hatfield and Colburn) hold patents that offer possibilities for cancer treatment, prevention or risk assessment. Among the former trainees of LCP PIs, seven are currently Assistant Professors in University Medical Schools and ten are Associate or Full Professors while four hold positions as Director or CEO of companies. LCP Postdoctoral and Predoctoral Fellows as well as students have won numerous travel awards and prizes for posters.

Since 2000 LCP PI's have been recognized as leaders in their fields in a number of ways. Dr. Colburn has organized a Keystone Conference and two AACR Symposia on Molecular Targets for Cancer Prevention. Dr. Ruscetti organizes an annual international workshop on retroviral pathogenesis. Dr. Hatfield has presented keynote lectures at major international conferences in Russia and India and has had a PNAS article featured in a commentary in PNAS and articles featured twice in CCR Frontiers in Science. Dr. Lanza serves as Co-investigator on multiple grants focused on colon cancer prevention. Dr. Ali has presented her research at major international meetings and had an article featured in Cancer Research Highlights. Drs. Lanza, Ali, and Colburn have presented invited talks or chaired workshops regularly at the AACR's annual Frontiers in Cancer Prevention Research meeting.

Among the contributions of LCP PI's to the CCR and the NIH is Dr. Ruscetti's service on the CCR Tenure Review Board and the Intramural Advisory Board and as Chair of the NCI Frederick Faculty Seminar Series. Dr. Colburn Chairs the NCI Frederick Distinguished Scientist Seminar Series and the Cancer Prevention Faculty, and serves on the Promotion Review Panel and the Steering Committee of the new Inflammation and Cancer Initiative. Dr. Lanza co-organized the 2003 Prevention Faculty Retreat. Dr. Colburn co-organized the 2005 Joint Retreat of the Breast and Gynecologic Cancer, Mouse Mammary Models and Cancer Prevention Faculties

Outside Funding: Several LCP Fellows have received outside support in the recent period. These include DCP supported Cancer Prevention Fellows mentored by Drs. Lanza and Hatfield, a National Agricultural Research Organization (NARO) Fellow mentored by Dr. Ruscetti, a Fellow supported by the Seoul National University in Dr. Hatfield's laboratory, a Japan Society for Promotion of Science Fellow mentored by Dr. Colburn, a Department of Energy Fellow, a Drug Discovery Fellow (CCR-Molecular Targets Development Program), and several International Union Against Cancer (UICC) Fellows in the laboratory of Dr. Colburn. Dr. Muegge was awarded a competitive Intramural Research Award to support a Fellow. Dr. Ali's budget is entirely supported by the Division of Cancer Prevention where she holds her primary appointment. Dr. Ali's laboratory and office space

are supplied by the CCR and are located in Bldg 469 in Frederick. Dr. Lanza's research is supported by a combination of grants and contracts.

Office of the Chief Support: In addition to secretaries Annie Rogers and Connie Champion GRS technical laboratory manager Glenn Hegamyer devotes 25% of his time to support budget, space, and equipment management for all LCP investigators.

Vision for the Future of the LCP: While we now represent a spectrum from molecular biology to mouse models to clinical prevention trials, we hope in time to achieve a continuum having several seamless interfaces. To bridge the gap between a tumor-inducing molecular event and clinical cancer prevention calls for more than screening human samples for gene mutations. Altered gene expression may be more important than gene mutation in driving the rate-limiting stages of carcinogenesis. Epigenetics is an area of strength in the LCP and we expect it to grow. Signal transduction research in the LCP offers insight into both activation and inactivation of oncogenes and tumor suppressors, key junctures for intervention. The ongoing studies of transcriptional, translational, and posttranslational regulation in the LCP are important for continuing to discover and characterize new molecular targets. Bridging to clinical trials calls for experimental designs that ask whether the expression of not one but a critical set of genes is modulated by an intervention under conditions in which cancer is prevented. Mouse models provide a powerful tool in which to connect short term readout of molecular target activation to cancer endpoints. Mouse model studies make possible a large number of variables in dose, combinations and timing of interventions that enable one to choose a small set of variables in a human trial. Querying mouse models for biomarkers found to be predictive in human samples is important as is the inverse. Several of us have become engaged in new translational initiatives that are rooted in a strong understanding of molecular and cellular biology. While we impact progress in the field by sending out dynamic new investigators into academia and industry, we also view as important the recruitment of new investigators to the LCP and the CCR to carry on the mission and realize the possibilities. In particular we would envision the addition of a tenure track scientist who would join the CCR's new initiative in Inflammation and Cancer. The ideal colleague might work with clinical prevention trials as well as with cell culture or mouse models focused on molecular targets for cancer prevention. Such recruitment efforts can bring to the CCR the seamless interfaces needed for successful research along a continuum from basic science to clinical trials.

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Principal Investigator: Nancy H. Colburn, Ph.D.

Scientific Overview: The major focus of the Gene Regulation Section (GRS) is to identify molecular events that drive the process of multistage carcinogenesis and to develop ways to target these molecular events for cancer prevention. The molecular events of greatest interest in the current period are AP-1 and NFκB dependent transcription, the subject of Project 1, and eIF4F/ Pdcd4 dependent translation, the subject of Project 2. Among the major achievements of Project 1 are the demonstration that targeting AP-1 and NFκB with dominant negative jun (TAM67) specifically inhibits not only DMBA-TPA induced skin carcinogenesis but also carcinogenesis induced by human relevant exposures, UVB and human papillomavirus E7. This period has also seen the first identification of TAM67 target genes that are functionally significant in tumor promotion. These target genes include high mobility group A1 (HMGA1) and cyclooxygenase-2 (COX-2). Molecular details of the activation of NFκB that are important in transformation have emerged, suggesting additional targets for cancer prevention. The Current Research and Future Plans of Project 1 are organized around five Specific Aims. The first and second Aims are directed to characterizing the regulation of identified targets of the AP-1/ NFκB inhibitor TAM67 and to identifying new targets of TAM67 that are functionally significant when TAM67 inhibits skin tumorigenesis without inhibiting growth or hyperplasia in the K14-TAM67/ K14-HPV E7 bitransgenic mouse model. The third Specific Aim is to develop and implement a high throughput screening assay to identify small molecule natural products that show the same specificity as the gene inhibitor TAM67. The fourth Aim is to determine whether TAM67 has preventive efficacy against tumorigenesis in other cancer sites, particularly colon, mammary gland and lung. The fifth Aim is to develop and use imaging, expression microarray and proteomics assays for monitoring the activation of proinflammatory molecular targets in intervention studies. Aim five will involve LCP and DCP collaborators in running parallel inquiries in mouse models and human clinical prevention studies.

Among the major achievements of Project 2 are to unravel the mechanism by which the novel transformation suppressor Pdcd4, discovered using the mouse JB6 model, works to inhibit transformation and tumorigenesis. Pdcd4 interacts with translation initiation factors eIF4A and eIF4G to selectively inhibit translation, to selectively inhibit AP-1 dependent transcription, to inhibit transformation, tumorigenesis and tumor progression. Pdcd4 inhibits tumorigenesis and tumor progression without inhibiting hyperplasia or inducing apoptosis. Although others have shown Pdcd4 expression to be upregulated by apoptosis inducers and cyclooxygenase-2 inhibitors, Pdcd4's possible function in the corresponding processes is not clear. Translational targets of Pdcd4 that are important in driving tumorigenesis or tumor progression need to be identified. The Current Research and Future Plans of Project 2 focus on five Specific Aims. The first is to identify functionally significant translational targets of Pdcd4. This uses four approaches to find mRNAs that have structured 5'UTR's, that are sensitive to Pdcd4 inhibited translation, that show reduced abundance in heavy polysomes and that are active in driving tumorigenesis or tumor progression. The second Aim is to ascertain how Pdcd4 expression is downregulated during multistage carcinogenesis, thus enabling the possibility of upregulating Pdcd4 for cancer prevention or intervention. The third Aim is to determine the functions both with and without carcinogenic challenge, of endogenous Pdcd4 using recently generated Pdcd4 null mice. The fourth is to determine molecular interactions of Pdcd4 with mRNA and translation initiation factors that function to regulate translation. This employs *in vitro* molecular interaction studies and X-ray crystallography. The fifth Aim is to identify targets in common between Pdcd4 and two other inhibitors of translation, mTOR inhibitor and immunosuppressant rapamycin, and antisense constructs of the pro-metastatic ezrin. Knowing the common targets may set the stage for discovery of drugs that target translation without targeting T cells.

Administrative Overview: During the period 2001-2005 George Washington University-NIH predoctoral student Halina Zakowicz completed her PhD and began a postdoctoral fellowship at the University of Wisconsin. Drs. Hsin-Sheng Yang, Jing Hu, Arindam Dhar, Myung Cho (sabbatical), Kazumi Suzukawa, and Tin-Chen Hsu completed postdoctoral fellowships. Drs. Yang, Hu and Suzukawa have begun positions as tenure track Assistant Professors at the University of Kentucky, the University of Pittsburgh, and the University of Tsukuba, Japan, respectively. Dr. Hu was awarded a K22 Grant to take with her. Dr. Yang received an Exceptional Capability Award in 2005 and an NIH FARE award in 2002. Dr. Dhar was chosen for the new NCI-FDA fellowship in 2005. He received travel awards for AACR Frontiers in Cancer Prevention and Annual meetings and had one of his publications featured in *CCR Frontiers in Science*. Dr. George Beck, an NCI Scholar also mentored by me, has begun a tenure track position as Assistant Professor at Emory University Medical School. He too brings a K22 Award with him. Staff Scientist Dr. Matthew Young was invited to speak at a 2003 IBS Meeting on Transcription Factors as Targets, at the Institute for Cancer Research in London by Dr. Christopher Marshall in 2003, and at a Molecular Targets for Nutritional Intervention Conference organized by the Division of Cancer Prevention, NCI in 2005. In 2003 the GRS was awarded a Johns Hopkins-NCI Drug Discovery Fellow Ms Katie Ruocco. Former GRS postdoctoral fellows have been promoted during this period: Dr. Yi Sun (l990-1995), to Professor at the University of Michigan Medical School and Dr. Jian-Jian Li (1995-1998) to Associate Professor at Purdue University. Dr. Zigang Dong (1991-1995), as Knowlton Professor and Director, Hormel Institute, University of Minnesota, is now among the leaders in signal transduction pathways as targets for cancer prevention.

I have served as organizer or co-organizer of conferences and symposia on Molecular Targets for Cancer Prevention at Iowa State University in 2002, at AACR Annual Meetings in 2003 and 2005 and at a Keystone Conference in 2006. I spoke at an International Conference on AP-1 Transcription Factors in 2003 and have been invited to speak as a "Distinguished Lecturer" or keynote speaker at MD Anderson Cancer Center, Houston and Science Park in 2004 and at a Congress on Molecular Staging of Cancer in Mannheim, Germany in 2006. In 2002 I received the NIH Merit Award and the NCI Outstanding Mentor Award and in 2003, was appointed to the Senior Biomedical Research Service and to the position of Chief, Laboratory of Cancer Prevention, CCR, NCI.

Principle Investigator: Iqbal U. Ali, Ph.D.

Scientific Overview: My laboratory was created as a bridge between the National Cancer Institute's Division of Cancer Prevention and the intramural program of NCI. It formally became a section of the Laboratory of Cancer Prevention in September 2004. The mandate of the Molecular Prevention Section is to develop molecular approaches to cancer prevention with an emphasis on identifying molecular targets of commonly used chemopreventive agents. We started out with a major focus on colorectal cancer – the third most prevalent and the second leading cause of cancer deaths in the United States. Colorectal carcinogenesis involves multiple genetic alterations and environmental exposures spanning over a long period of time providing opportunities for prevention. Genetic and pharmacological evidence has suggested that the enzyme cyclooxygenase 2 (COX-2) is a promising target at the stage of adenoma development. Both selective and non-selective inhibitors of COX-2 have shown efficacy in clinical trials. Taking advantage of some of these clinical resources, and using integrated genomic and proteomic approaches, we have focused on COX-2 and its inhibitors and developed a comprehensive program:

1. To understand the influence of genetic variants of COX-2 on adenoma risk,
2. To search for molecular targets modulated by a widely used selective inhibitor of COX-2, celecoxib, and
3. To understand the molecular basis of variable response of patients to celecoxib.

We have previously shown that association studies can help explore gene-environment interactions and identify subgroups of individuals most likely to benefit from specific interventions [1-3]. A similar approach has suggested that polymorphisms in the *COX-2* gene modify the risk of adenoma development and the protective effect of non-steroidal anti-inflammatory drugs (NSAIDs) [4]. Current work and future plans include an extensive analysis of *COX-2* haplotypes for association with adenoma risk and pharmocogenetic interactions with NSAIDs in randomized screening and chemoprevention trials.

We identified a panel of specific proteomic markers in serum samples from the prevention clinical trial of familial adenomatous polyposis (FAP) patients that were significantly modulated by celecoxib [5]. Current work is directed toward identifying and characterizing celecoxib-modulated molecular targets in clinical specimens and in colorectal cancer cell lines by a combination of transcriptomic and/or proteomic approaches. Additionally, proteomic profiling of sera and nipple aspirates from a breast cancer prevention trial using celecoxib would determine if celecoxib modulation transcends gender, organ site, and duration of treatment.

A significant step toward the goal of individualized prevention/treatment would be an understanding of the molecular basis of patients' variable response to prevention/intervention strategies. By using multiple classification algorithms, we have identified a serum proteomic marker of *m/z* 16961 capable of predicting response/non-response of FAP patients to celecoxib with high sensitivity and specificity [6]. Future plans include validation of this response classifier in a larger study of FAP patients treated with celecoxib as well as its identification and characterization by conventional protein chemistry techniques.

In a second project, we are using multiple proteomic techniques to study lung cancer, the leading cause of cancer deaths worldwide. Temporal profiling of plasma samples from lung cancer patients identified diagnostic spectral features that were present in the plasma several years before death [7]. Current work and future plans focus on identifying diagnostic markers for lung cancer that may

signal the onset of the disease much before the appearance of clinical symptoms and would contribute toward developing meaningful prevention/intervention strategies.

Administrative Overview: I moved to my own laboratory space in April 2004; currently two scientists and one technician are working under my supervision. Initially, the first post-doc was hired in summer of 2001, who worked under my direction for <3 years in collaboration with the Mass Spec Core Facility. During this short period of time, our work has produced significant results that attracted much attention. At the Cancer Prevention Meeting in 2003, my collaborator, Iman Hakim, the PI of the SEATS intervention clinical trial, participated in the AACR press conference regarding our work on the significance of genetic polymorphisms in determining the outcome of intervention by the antioxidants in tea polyphenols. Our work on the identification of response predictors to celecoxib generated considerable interest. The article was featured in "Cancer Research Highlights" in the April 15, 2004 issue of Cancer Research and NCI issued a press release entitled "Proteomics Shows Promise in Colon Cancer Chemoprevention Study." http://www.cancer.gov/newscenter/doc.aspx?viewid=07A5FF5E-E140-4D80-9C94--7DD9FA67C8F. I also received performance awards from the Division of Cancer Prevention on my work in 2004 and 2005. I presented my work at two international meetings, Proteomic Forum 2003 in Muenchen and HUPO 2nd Annual Congress 2003 in Montreal. I was invited to chair a session at the 2nd International Conference "Genomics, Proteomics and Bioinformatics for Medicine" in 2004 in Moscow and to give two talks in Genomics and Proteomics sessions. I was an invited speaker at the Genomics and Proteomics Interest Groups at the George Washington Cancer Center in March 2004 to present my work on proteomics applications in cancer prevention and in December 2004 to teach a course to graduate students on association studies using genotypes versus haplotypes. I gave invited presentations for the Proteomics Interest Group of NIH in May of 2004 and at the Howard University Cancer Center in July 2004.

My program uses clinical resources from the DCP-funded clinical trials. I successfully competed and obtained 1600 samples for the colorectal adenoma study and 3000 samples for the prostate carcinoma study from the PLCO screening trial. Additionally, I have ongoing/future collaborations with Drs. Robin Phillips (St. Mark's Hospital) and Patrick Lynch (MD Anderson) on two FAP trials, John Baron (Dartmouth Medical Center) on the adenoma recurrence study, Ed Sauter (University of Missouri) on the breast cancer study, and Elaine Lanza (LCP) on the PPT trial. I extensively use and collaborate with scientists at NCI's Core Service Laboratories in Frederick, i.e. Laboratory of Molecular Technology headed by Dr. David Munroe and Laboratory of Proteomics and Analytical Technologies headed by Dr. Timothy Veenstra. I have collaborations with Drs. Brian Luke, Ming Yi, and Robert Stevens of the Advanced Biomedical Computing Center, SAIC for all the bioinformatics work involved in my projects. Since setting up my new laboratory, I trained a post-doctoral fellow, Zhen Xiao, who has moved up to Scientist I position in the Laboratory of Proteomics and Analytical technologies at SAIC. I mentored a student, Wali Malik, from NCI's Cancer Research Intern in Residence program in the summer of 2004, who is now majoring in molecular genetics at the University of Maryland.

Principal Investigator: Dolph L. Hatfield, Ph.D.

Scientific Overview: Selenium is an essential micronutrient in the diet of humans and other mammals. This trace element has been reported to be an important cancer chemopreventive agent [1], to inhibit viral expression [2], delay the progression of AIDS in HIV positive patients [3], slow the aging process [4], prevent heart disease and other cardiovascular and muscle disorders [5] and to have roles in mammalian development [6, 7], male reproduction [8] and immune function [9]. There has been considerable debate in the selenium field whether low molecular weight selenocompounds or selenium-containing proteins, or both, are responsible for these health benefits [10]. One of our major hypotheses has been that selenoproteins play an essential role in development and mediate many of the health benefits of selenium (reviewed in Hatfield and Gladyshev [11]).

The steps involved in the biosynthesis of the selenium-containing amino acid, selenocysteine (Sec), and its incorporation into protein have been thoroughly defined in bacteria [12]. However, the comparable pathways have not been fully resolved in mammalian cells due to the complexity of the components and regulatory mechanisms involved. For example, in mammals, there are two major isoforms of Sec $tRNA^{[Ser]Sec}$ that differ by a single 2'0-methyl group on the ribosyl moiety at position 34 [11] (designated Um34). We have shown that the two Sec $tRNA^{[Ser]Sec}$ isoforms are involved in the expression of different selenoproteins [13, 14] confirming our earlier, key hypothesis that these two isoforms are utilized differently in selenoprotein synthesis [15]. This observation, however, raises an important question which is how can Um34 modulate the synthesis of only certain selenoproteins? In addition, there are other factors such as SECp43 [16] and the soluble liver antigen (SLA) [17] that form a complex with Sec $tRNA^{[Ser]Sec}$, and phosphoseryl-$tRNA^{[Ser]Sec}$ kinase (PSTK) [18], but their roles are poorly defined. One of the major interests of the MBSS is the means by which Sec is biosynthesized and inserted into protein in mammals that we propose must involve each of these factors.

The goals of the Molecular Biology of Selenium Section (MBSS), therefore, fall into two broad areas: 1) preparation and utilization of mouse models for assessing the roles of selenium and selenoproteins in development and health including cancer prevention and other maladies; and 2) determination of the mechanism of Sec biosynthesis and its incorporation into protein.

To assess the roles of selenium and selenoproteins in development and health, we have generated the following mouse models: i) two different mutant Sec $tRNA^{[Ser]Sec}$ transgenic mouse lines encoding either a A->G mutation at position 37 [19] or a T->A mutation at position 34 [20], ii) a conditional knockout mouse line involving the Sec $tRNA^{[Ser]Sec}$ gene (designated *trsp*) [7], and iii) a mutant *trsp* transgenic/*trsp* knockout mouse line [13, 14] that can be used to target individual tissues and organs [20]. These mouse models have taken advantage of the fact that selenoprotein expression is unique in that this class of proteins is dependent on the presence of Sec $tRNA^{[Ser]Sec}$; and thus by perturbing the expression of this tRNA, the synthesis of different selenoproteins, as well as selenoproteins as a whole, can be depleted or modulated in specific tissues and organs. We have targeted, or are targeting, *trsp* knockout in breast, liver, endothelial cells, skeletal and heart muscle, T-lymphocytes, macrophages, prostate, colon and neurons (summarized in Table 3). Our demonstration that the targeted removal of selenoprotein expression in endothelial cells causes embryonic death [21] and in heart muscle causes cardiac failure [21] further substantiates our hypothesis that selenoproteins play a key role in development and disease prevention. We are studying in detail the effects of selenoprotein loss in prostate, breast, colon and neurons on a collaborative basis while emphasizing the role of selenoproteins in immune function in our laboratory. Our aim is to assess how quickly lesions arise in prostate, breast and colon lacking selenoprotein expression following exposure of the corresponding mice to a carcinogen and/or a cancer driver gene. Following a complete

characterization of T-cell and macrophage phenotypes, we plan to challenge the immune system of these mice with stresses including viral and bacterial pathogens, carcinogens and tumor implantation/rejection studies.

As noted above, we have now shown that the two Sec $tRNA^{[Ser]Sec}$ isoforms (designated mcm^5U [methylcarboxymethyl-5'-uridine] and mcm^5Um [methylcarboxymethyl-5'-uridine-2'O-methylribose]) have different roles in selenoprotein expression [13, 14]. We therefore propose to elucidate the pathway of Sec biosynthesis and the role of mcm^5U in synthesizing housekeeping selenoproteins and mcm^5Um in synthesizing stress-related selenoproteins. In addition, we have isolated and characterized PSTK [18] and have shown that SECp43 has a role in Um34 synthesis [22]. We have shown that SLA forms a complex with SECp43 and that the latter factor likely serves as a chaperone in shuttling SLA and Sec $tRNA^{[Ser]Sec}$ into the nucleus. We plan to further explore the role of SLA along with those of other proteins involved in selenium metabolism including SECp43, SPS1 and 2 and PSTK to elucidate the pathway of Sec biosynthesis and its incorporation into protein.

The MBSS, in collaboration with Dr. Vadim Gladyshev, were the first to show that eukaryotic organisms outside the animal kingdom utilize the Sec insertion machinery by demonstrating that *Chlamydononas reinhardtii*, a lower plant, encodes 10 selenoprotein genes in its genome [23]. In addition, we have developed simple approaches of defining Sec tRNA genes by a computational technique and sequencing Sec tRNAs by RT-PCR [24, 25]. We sequenced the first three eukaryotic Sec tRNAs outside the animal kingdom in the model organisms, *C. reinhardtii* [24], *Tetrahymena thermophila* and *Dictyostelium discoideum* [25]. We plan to pursue, on a limited basis, the occurrence of Sec tRNA in numerous other model, eukaryotic organisms to assess how widespread the use of the Sec insertion machinery is among eukaryotes.

Since one of the key priorities of the LCP is cancer prevention and a major aim of the MBSS is to determine how selenium is effective in preventing cancer and other maladies, the research of the MBSS contributes significantly to the overall goals of the lab.

The MBSS has several intra- and extramural collaborations that are given in Table 1.

Principal Investigator: Elaine Lanza, Ph.D.

Scientific Overview: The effect of nutrition on colorectal cancer (CRC) prevention is the main focus of my projects and, like all cancer prevention research, demands the integration of biological, epidemiological, and behavioral disciplines to identify efficacious and applicable interventions [1]. Findings from epidemiology can be tested in interventions in animal models to study the entire carcinogenesis process, biomarkers and molecular targets, and to identify the impact of interventions on the entire biosystem. However, recognizing rodent and other animal model limitations to human prevention, clinical nutrition studies must also be conducted [2]. Human controlled feeding studies can improve our understanding of the effects of dietary interventions on metabolism and biomarkers of disease susceptibility. These studies provide an evaluation of the plausibility of hypotheses generated from observational studies, identify biomarkers associated with dietary changes, and serve as pilot studies for larger scale intervention trials [3].

Project 1 described in this site visit report focuses on the role of dried beans on colorectal cancer risk. Although, there was no effect of the Polyp Prevention Trial (PPT) intervention diet on adenoma recurrence [4], I explored whether changes in particular categories of fruit and vegetable intakes in the PPT were associated with reduced adenoma recurrence. This analysis considered both intervention adherence and the multitude of phytochemical changes made by trial participants. I found that the highest quartile of change in dried bean intake, median 42g/d (three times the U.S. level of intake) compared to those in the lowest quartile had a significantly reduced odds ratio for advanced adenoma recurrence [5]. To further investigate this finding, I initiated two research studies: a controlled feeding study on the effects of a high dried bean low glycemic index diet on insulin resistance and inflammation in patients at high risk for colorectal adenoma recurrence, and an animal carcinogenesis study in ob/ob mice.

A controlled feeding study in 68 men with a history of adenomas, with and without insulin resistance, is being conducted to understand whether dried beans decrease acute phase proteins, such as C-reactive protein, and circulating inflammatory cytokines, as well as markers of insulin resistance, such as C-peptide. In this study, I am also examining alterations in gene expression in fecal colonocytes. The effects of diet on gene expression in these exfoliated cells should provide insights into dietary induced changes in genes associated with the insulin and inflammatory pathway in the colonic epithelium. The animal study on the effects of dried beans in azoxymethane (AOM) induced colon cancers in C57BL/6J *ob/ob* mice, a model of genetic obesity, is being conducted in male and female mice. The animals are being fed whole navy beans and two navy bean fractions, the phytochemical fraction and the dietary fiber residue, to provide insight into the anti-cancer potential of each component.

Project 2 explores the role of inflammation, obesity, and insulin resistance in colon cancer and the effect of diet in mediating these risks. The results from clinical trials and observational studies generally support an inverse association between NSAID use and colorectal adenoma recurrence. However, the reduction in risk is generally less that 50% [6], indicating that many individuals are non-responders to these agents. Therefore, I explored the interaction between NSAIDs use and the PPT intervention diet to investigate whether certain diets modify and/or antagonize the protective effects of NSAIDs. NSAIDs modified the association between the intervention and adenoma recurrence at baseline ($p=0.02$) and throughout the trial ($p=0.008$). The results suggest that adopting a low-fat, high-fiber diet rich in fruits and vegetables may lower the risk of colorectal adenoma recurrence among individuals who do not regularly use NSAIDS [7].

Dissecting interactions between complex genetic traits and lifestyle characteristics to identify risk factors for colorectal cancer remains a significant challenge for reducing the burden of this disease. It is possible that single nucleotide polymorphisms (SNPs) may play a role in the development of colorectal adenomas. In the PPT cohort, I am investigating interactions between genotypes/haplotypes and lifestyle/dietary factors in inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10), DNA repair (ERCC2, ERCC4, OGG1, XRCC1, XRCC3), obesity/behavior (COMT, DRD2, HTR1B, GAD2) and Gpx1.

Obesity increases inflammation and is an emerging risk factor for colon cancer [8]. Insulin resistance (IR) and type 2 diabetes are also emerging as significant risk factors for colorectal cancer (CRC) [9] and adenomas [10], and insulin resistance is recognized as chronic, low-level, inflammatory state [11]. I am exploring whether inflammatory cytokines and/or biomarkers associated with obesity and insulin resistance, influence adenoma recurrence in the PPT.

Although there was no effect of a comprehensive dietary intervention on the recurrence of colorectal adenomas during the four years of the PPT, it was hypothesized that a statistically significant intervention effect may only emerge after the four years of the trial. In the PPT Continued Follow-up Study, we collected an additional 4 years of post-intervention follow-up data on 801 participants to address this question. Although there was still no significant difference in recurrence rates in the intervention and control groups, this data set provides the opportunity to address a number of clinical issues with respect to colonoscopy surveillance and adenoma recurrence patterns. I continue to study modifiable lifestyle [12, 13] and genetic risk factors for adenoma recurrence and I am involved in three collaborative projects on the recurrence of adenomas and advanced adenomas [14, 15].

Knowing what dietary factors are associated with cancer risk reduction is important, but not sufficient for cancer prevention. Research clearly suggests that dietary changes are difficult both to initiate and maintain. Therefore, I am investigating factors associated with dietary compliance in the PPT [16]. I am also exploring the effects of genetic variability in reward-motivated behavior, which may alter dietary compliance and thus the risk of being overweight and/or obese. Applications of these findings are widespread for public health nutrition and obesity intervention programs.

Administrative Overview: I have been a Principal Investigator at NCI since 1988. In January 2003, I joined the Basic Research Laboratory and in April 2004, moved to the newly formed Laboratory of Cancer Prevention. Since my last Site Visit, I have mentored several Cancer Prevention Fellows: Drs. Leah Sansbury, Marie Cantwell, Kay Wanke, Tamaro Hudson, Connie Rogers, Ken Hance and Michel Dixon who completed his Ph.D. thesis in Nutrition at Howard University, Washington DC under my direction. I have been a member of the Cancer Prevention Faculty Steering Committee since 2001.

Principal Investigator: Chou-Chi H. Li, Ph.D.

Overview: VCP, also known as p97, Cdc48, Ter94, VAT, etc. in different organisms, belongs to an ancient protein family named AAA (ATPases associated with a variety of cell activities). The 97-kDa VCP (p97-VCP or VCP) is an essential, abundant, and highly conserved protein. Despite the high sequence and structural conservation, VCP and its orthologs have been implicated in a wide variety of seemingly unrelated cell activities, suggesting that it carries out a basic function that underlies all the activities (reviewed in ref. 7, 11, please also see the cited ref. in them).

We previously showed that VCP physically associates with many substrates of the ubiquitin-proteasome (Ub-Pr) degradation pathway, including NF-κB proteins c-Rel (3) and IκBα (1), cell cycle proteins cyclin (2) and Plk (4). During this review period, we further reported that VCP associates with a number of non-tyrosine kinase-type cytokine surface receptors, which are also subject to Ub-Pr regulation (5). This seemingly promiscuous binding of VCP to the various Ub-Pr substrates suggests that VCP may be a missing link between the Ub-conjugates and the proteasome, an intensively investigated area in the Ub-Pr research field. Indeed, our study provided the first evidence that VCP physically associates with the 26S proteasome (1) and many ubiquitinated protein substrates, and is required for the Ub-Pr-mediated degradation (6). The binding between VCP and the substrate is directly mediated through the Ub chains of the substrate and the N-terminal domain of VCP (6). Therefore, VCP acts as a molecular chaperone that specifically binds the poly-ubiquitinated substrates and targets them to the proteasome for degradation, hence indirectly regulates the diverse cellular activities through the Ub-Pr pathway,.

VCP molecule can be divided into four major domains: the N-terminal 200 residues, named N domain, two conserved ATPase domains D1 and D2, and the C-terminal region (reviewed in ref.11). VCP has a barrel-like structure containing two stacked homo-hexameric rings made of the respective D1 and D2 domains. Using *in vitro* degradation assays, we showed that in addition to the N domain, both D1 and D2 are also required to mediate the Ub-Pr degradation (8, 9). Presumably, either or both of the ATPase domains hydrolyze ATP, which fuels the chaperone activity of VCP. In a biochemical study, we systematically characterized the ATPase activity of VCP and reported the novel finding that the two ATPase domains are not catalytically equal: D2 is responsible for the major enzyme activity at physiological temperature, whereas D1 mediates a heat-enhanced ATPase activity (9) .

VCP has an unusually high preponderance to form a hexameric structure, which is essential for its biological functions. We showed that unlike most of the AAA family proteins that require the presence of nucleotide to form oligomers, VCP readily form homo-hexamers in the absence of nucleotide (8, 10). This nucleotide-independent oligomerization requires the D1 domain and the linker sequence between D1 and D2 (10). During ATPase cycles, VCP undergoes conformational changes that presumably apply tensions to the bound substrate, leading to the disassembly of protein complexes or unfolding of the substrate. How ATPase activity is coupled with the conformational changes in the VCP hexamer is not clear. We took biochemical approaches to study the structure of VCP in different nucleotide conditions to depict the conformational changes during the ATPase cycles (10). Based on our data, we proposed a model whereby the D1 ring is relatively stable and undergoes minor conformational changes; but the D2 ring undergoes pronounced conformational changes during the ATPase cycle (10). The D2 ring exhibits a compact conformation when bound to nucleotide, but adopts a much more open and flexible conformation[5] after ADP is released at the end of the ATP hydrolysis (10). This model agrees with the subsequently reported crystallography study (14, 15).

Recently, we further studied the significance of the Second Region of Homology (SRH) which is a highly conserved motif in AAA module (12). Our studies show that the conserved Arg residues in the SRH of both D1 and D2 domains play a critical role in communicating the conformational changes coupled with ATP hydrolysis, and the SRH in D1 also contributes to substrate binding and cofactor communications (12).

Our current and future studies focus on the chaperone activities of VCP and the structural requirements for such activities, specifically in the aspects of 1) prevention of protein aggregate formation (13), 2) disassembly of protein-protein complexes, and 3) modification of protein conformation. Recently, genetic studies identified specific VCP mutations as the pathological cause for a dominant lethal disease, Inclusion Body Myopathy associated with Paget disease of bone and Frontotemporal Dementia (IBMPFD) (19). In collaboration with Dr. V. Kimonis, we have started to examine the pathogenesis of VCP malfunctions (mutations and abnormal expressions) in human diseases, including cancers, IBMPFD and neuro-degenerative diseases, in which the Ub-Pr pathway has been shown to play an important role. Furthermore, through collaborative effort with the NCI core facilities, we plan to explore the possibility of developing VCP as a therapeutic target for human diseases.

Principal Investigator: Kathrin Muegge, Ph.D.

Overview: Packaging of DNA into chromatin organizes the genome into 'active 'zones of euchromatin and 'repressed zones of 'heterochomatin'. These opposing states of chromatin compaction ultimately control the access of DNA binding factors to their specific target sites. This can regulate a number of DNA based processes such as transcription, recombination or DNA repair. Chromatin states can be inherited by the next cell generation, thus preserving specific gene expression pattern, a phenomen known as epigenesis. Epigenetic modifications of chromatin are largely determined by specific posttranslational modifications of histone tails or methylation of cytosine residues. A disturbance of epigenetic patterns plays a role in human inherited diseases (such as the RETT or ICF syndromes) and is thought to play a role in cancer development.

To understand more about the molecular mechanisms of epigenesis it is important to study the molecular pathways that determine chromatin structure. Our laboratory has identified Lsh as a major epigenetic regulator which controls DNA methylation patterns and is crucial for normal heterochromatin formation in mice. By using Lsh-/- mice as a genetic tool to dissect some of the pathways that shape chromatin, we hope to gain more insights into the role of chromatin during normal development and tumorigenesis.

Previously, this laboratory has identified and cloned Lsh (lymphoid specific helicase) from a lymphoid precursor cell library and characterized Lsh's genomic structure and its presence in different species [1, 2]. Lsh is preferential expressed in the thymus and testis in adult mice and ubiquitously expressed in embryonic tissues. Lsh belongs to a subfamily of helicases, known as SNF2 family whose members participate in SWI/SNF chromatin remodeling complexes. SNF2 homologues are thought to be the engines that require ATP for nucleosomal remodeling e.g. by sliding nucleosomes along DNA and thus modulating access of other DNA binding factors. Based on its homology to SNF2 members we hypothesize that Lsh has a presumed chromatin remodeling activity. Our main interest has been to reveal the molecular effects of Lsh on chromatin formation and to understand the biologic consequences of epigenetic modifications caused by Lsh.

Principal Investigator: Sandra K. Ruscetti, Ph.D.

Scientific Overview: The Retroviral Molecular Pathogenesis Section of the LCP uses retroviruses to understand the molecular basis for various diseases. We have been studying retroviruses that cause leukemia or neurological disease in rodents to obtain basic information on how molecular changes in normal cells result in pathological consequences. By providing important information about the cause of disease, these animal models are invaluable for identifying and validating molecular targets relevant to human cancers for their prevention and treatment, the main research goal of the LCP.

The major focus of our research is to understand the molecular basis for the erythroleukemia induced by the Friend spleen focus-forming virus (SFFV), which provides an important model for understanding how deregulation of hematopoietic pathways can lead to leukemia [1]. The growth and differentiation of an erythroid cell is a highly regulated process that requires binding of erythropoietin (Epo) to its cell surface receptor to activate signal transduction pathways. However, erythroid cells infected with SFFV proliferate and differentiate in the absence of Epo, resulting in an acute erythroid hyperplasia that represents the first stage of the disease. We previously demonstrated that the unique envelope protein encoded by SFFV, gp55, is responsible for these early biological effects [2] and that the induction of Epo-independence by the virus is the result of constitutive activation of various Epo signal transduction pathways [3-6]. During the current review period, we have made important advances in understanding how signal transduction molecules are activated in SFFV-infected cells. We demonstrated that SFFV gp55 interacts with and activates a short form of the receptor tyrosine kinase Stk, termed sf-Stk [7], whose expression and kinase activity are essential for the induction of Epo-independence by SFFV [8, 9]. We further showed that SFFV-infected erythroid cells continue to express high levels of the anti-apoptotic protein Bcl-X_L after Epo withdrawal, promoting their survival [10]. We also made the novel observation that co-expression of SFFV gp55 and sf-Stk can transform rodent fibroblasts in the absence of the Epo receptor and can cause cancers in mice that had not previously been associated with SFFV infection [11]. In addition to causing Epo-independent erythroid hyperplasia, SFFV can also transform erythroid cells by integrating into the *sfpi-1* locus, stimulating expression of the myeloid transcription factor PU.1 and blocking erythroid differentiation. We demonstrated that immortal murine erythroleukemia (MEL) cells resulting from this secondary genetic change are blocked in STAT1 phosphorylation and DNA-binding activity induced by Epo or SFFV, but not by interferons, and express high levels of the hematopoietic phosphatase SHP-1 [12]. We also demonstrated that a pharmacological inhibitor of Jun-N-terminal kinase (JNK) blocks the proliferation and survival of SFFV MEL cells, suggesting that JNK may be a valid molecular target for therapeutic intervention [13]. Finally, we have shown that SFFV MEL cells metastasize to the bone marrow, where they are retained and cause meningeal leukemia. Our goals for the next review period are to understand how co-expression of SFFV gp55 with sf-Stk results in the constitutive activation of signal transduction molecules in both erythroid and non-erythoid cells; to determine the molecular basis for the block in erythroid cell differentiation and transformation of SFFV-infected erythroid cells as well as their retention in the bone marrow during the second stage of the disease; to devise specific therapeutic strategies to treat both stages of SFFV-induced erythroleukemia; and to ascertain whether sf-Stk or a short form of RON, its human analogue, plays a role in any rodent or human cancers.

As a second retroviral model system, we have been studying PVC-211 murine leukemia virus (MuLV), a variant of the erythroleukemia-inducing Friend MuLV that causes a rapid neurodegenerative disease in rodents [14]. PVC-211 MuLV provides an important model for understanding how retroviruses can undergo genetic changes that alter their interaction with cells in

the host to cause novel biological effects. We previously demonstrated [15, 16] that PVC-211 MuLV has undergone subtle genetic changes in its envelope gene, allowing it to efficiently infect brain capillary endothelial cells (BCEC), which are generally resistant to infection by MuLVs. This expanded host range allows PVC-211 MuLV to be expressed at high levels in the neonatal rodent brain, resulting in rapid neurodegenerative disease. During the current review period, we have made important advances in understanding the BCEC tropism of PVC-211 MuLV and identifying changes that occur in virus-infected BCEC that may contribute to the death of neurons. Our data demonstrates that the envelope protein of PVC-211 MuLV contains a unique heparin-binding domain that may allow the virus to bind strongly to the surface of BCEC via heparin-like molecules, facilitating its interaction with its cell surface receptor [17]. By comparing BCEC from uninfected and PVC MuLV-infected rats, we found that virus-infected BCEC express high levels of inducible nitric oxide synthase (iNOS) and the chemokine LIX and show evidence of NO production [18]. Our goals for the next review period are to further characterize the molecular events in PVC-211 MuLV-infected BCEC leading to neurodegeneration and identify agents effective for the treatment or prevention of this disease.

Administrative Overview: In 2003, I was promoted to the Senior Biomedical Research Service, a career pathway for outstanding researchers at the NIH. During this review period, I have been invited to present our work at numerous national and international scientific meeting and workshops. I am actively involved with an international group of scientists with an interest in retroviral pathogenesis and help to organize and participate in an annual workshop on this topic. I am frequently asked to review articles for scientific journals and to evaluate scientists for promotion within their Institutions. At the NIH I have served on numerous committees, including the CCR Tenure Review Board and the CCR Intramural Advisory Board. In Frederick, I continue to organize the Frederick Faculty Seminar Series, which we initiated 8 years ago to promote better communication and collaborations among the research community at NCI-Frederick, and serve on the Conference Center Users Committee.

I have mentored several postdoctoral fellows during this review period who received awards and recognition for their research. In 2001 and 2002, Kazuo Nishigaki was chosen to receive an exceptional stipend increase, and in 2003 one of his scientific manuscripts was showcased as a Platinum Highlight Article in the *National Cancer Institute at Frederick Poster,* a monthly newsletter. Another postdoctoral fellow, Karen Rulli, received awards for her outstanding poster at the 15th International Workshop on Retroviral Pathogenesis in Glasgow, Scotland, and at the 2003 Spring Research Festival in Frederick. She also was awarded a Fellows Award for Research Excellence in 2003. All of the postdoctoral fellows that have completed their fellowships in my laboratory during this review period now hold scientific research or administrative positions at excellent research institutes or companies.