

ONGOING CENTER RESEARCH AND NEW INITIATIVES

Over the last 5 years, the Center has grown from 55 to 80 primary investigators. They have mutual interests in the Center research activities and contributed substantially to the Center's research base by participating in core laboratories, collaborative efforts with other Center investigators, and serving on Center committees. Although the Center investigators have diverse research goals and objectives they share a common thread interest on the biochemistry and physiology of the neurohormonal mediators of communication between different cells or organs of the body. Some investigators focus on basic molecular research while others concentrate on more integrated systems. Some work primarily on gastrointestinal research problems with only a peripheral interest in peptide hormones, while others work with gut peptides in non-gastroenteric organs. Over the course of the last 20 years, the Center has united its investigators together in a collaborative effort that has broadened the horizons of the individual laboratories and moreover, widened interest in digestive disease related research. The specific research interests of the Center investigators can be grouped in the following arenas:

1. Transgenics and Gene Expression Profiling

Many Center investigators take full advantage of the outstanding facilities and staff of the transgenic Core to develop new animal models. Not surprisingly, transgenic rodent technology has become the focal point of studies combining molecular and physiologic approaches. Almost half of current Center Members use transgenic or knockout models to examine physiological and pathophysiological roles of peptides and other regulatory molecules in health and disease. In addition, one third of the Center Members used the Transgenic Core in the past funding period to generate new rodent models. For example, the Cdx2-Cre mouse developed by the Fearon laboratory has been an important tool to study the significance of the gatekeeper APC gene in the colon (1). They developed a transgenic mouse with a *Cdx2* genomic fragment that drives Cre recombinase in the distal ileum and colon. When the Cdx2-Cre expressing mouse is crossed to the mouse model expressing the LoxP-flanked APC gene, adenomatous polyps were observed in the colon and not the small intestine. This model represents an important advance since numerous prior studies of the mutant mouse APC gene (i.e. APC^{min} mouse) results primarily in intestinal and rarely in colon polyps characteristically observed with the human mutant APC phenotype. Another series of studies conducted by Dr. Kaufman's group utilized mouse mutants with genetic deletions of endoplasmic reticulum (ER) stress response genes (2). The Transgenic Core assisted this group's generation of several genetically engineered mouse models, including a null mouse for the ATF6 α transcription factor (3) and a conditional floxed model of the mRNA splice regulator IRE1 (4). ATF6 α -deficient mice showed hepatic steatosis when challenged with inducers of ER stress (unfolded protein response, UPR), and liver-specific deletion of IRE1 demonstrated that the mechanism was mediated by direct regulation of metabolic gene expression. Thus this team of investigators directly linked ER stress to regulation of lipid homeostasis through UPR, demonstrating a critical link between this pathway and hepatic steatosis (4).

Viral vectors have been an important technology for gene transfer into primary cells isolated from the gastrointestinal tract and other cultured cell models. Twenty-five

different Peptide Center investigators have taken advantage of the Viral Vector Core for assistance with this technology (35% of the current membership). Of note is the strong increase in use of lentiviral vectors to infect neurons and differentiated gastrointestinal epithelial cells. For example, in a series of studies of Dr. Owyang, a lenti-virus-based system for delivery of small interfering RNA (siRNA) listed with GFP promoter was used to silence the expression of the 2 pore K⁺ channel TRESK which is upregulated in diabetic nodose ganglia. The Viral Vector Core has helped to design and construct the LV shRNA vectors. Transfected diabetic neurons reverted the electrophysiological characteristics of diabetic neurons to normal values. At the same time, in vivo electroporation of the nodose ganglia with TRESK siRNA restored the reduced pancreatic response to CCK stimulation in diabetic rats. These studies show that upregulation of the 2 pore K⁺ channel TRESK occurs in diabetes and this is likely to be responsible for many of the impaired GL functions which are mediated by vago-vagal reflex. As a result of the strong preliminary data presented, Dr. Owyang is able to successfully compete for a new R01 grant (DK 084039).

Gene expression profiling has become an essential tool to identify in an unbiased manner changes in gene expression associated with gastrointestinal diseases in addition to physiological regulation of development and function of gastrointestinal cells and tissues. Approximately 20% of Peptide Center Members have taken advantage of the Microarray Service in the past funding period. This technology has been used for both clinical and basic research. For example Dr. Simeone has used this technology to identify molecular signatures of pancreatic cancer from laser capture isolated tissues. Microarray analysis of pancreatic cancer samples identified marked elevation of ATDC (ataxia-telangiectasia group D complementing) gene expression in most invasive isolates as well as in precursor lesions, suggesting that this protein is a critical player in pancreatic cancer development (5). Further in vitro analysis showed that ATCD promoted cancer cell proliferation and in vivo analysis demonstrated enhanced tumor growth and metastasis through activation of the Wnt pathway. Thus the discoveries emerging from this study have led to the identification of possible new biomarkers that are critical effectors for pancreatic cancer. Dr. Samuleson has used gene expression microarrays to profile changes in genetic models disrupting normal parietal cell function. Gene expression profiling of gastrin-deficient and wild type mice demonstrated critical changes to parietal and ECL cells (6-7). This analysis demonstrated that gastrin is not required for formation of these cell types, but instead is essential for normal maturation and function of these gastric epithelial cells. Dr. Gumucio has used expression profiling to examine differential gene expression between stomach and intestine during embryonic development (8). This study compared mesenchymal and epithelial gene expression at the pyloric border to define critical genes associated with activation of organ-specific gene development. These studies have provided target genes for ongoing analysis of mechanisms relating to intestinal villus formation.

2. Signal Transduction

Center members carrying out studies of signal transduction continue to probe soluble intracellular messengers such as Ca²⁺ and cyclic nucleotides. Most recent studies, however, have involved quantitating phosphorylation of individual proteins by Western blotting to understand activation of specific signaling pathways. These studies have involved various protein kinase cascades such as the MAP Kinase cascades leading to ERKs, JNKs and p38 and the PI-3K-mTOR pathway leading to Akt, p70 S6K and translational control. Newer pathways involving Wnts, Hedgehog, and Notch are being

studied as they relate to differentiation. Many pathways involve both protein phosphorylation and protein-protein interaction. The study of protein phosphorylation is being extended by the use of immunohistochemistry and confocal microscopy to visualize which cells in a tissue show enhanced phosphorylation and by proteomics to evaluate the spectrum of proteins showing changes in phosphorylation.

A large group of Center Investigators are studying the role of signaling pathways in differentiated cells of the GI tract. These studies are focused on the pancreas (J. Williams, D. Simeone, C. Owyang, D. Sans, M. DiMagno, P. Dempsey, and P. Arvan), Gastric mucosa (A. Todisco, L. Samuelson, J. Merchant, A. Dlugosz), intestinal mucosa (K. Bitar, D. Gumucio, E. Zimmerman, D. Teitelbaum) and neuronal cells of the autonomic nervous system (C. Owyang, M. Mulholland, S. Morrison, J. Wylie) and hepatocytes (L. Rui, O. MacDougald, C. Burant). Interestingly, signaling is also being studied in specific brain nuclei as it relates to control of feeding and body weight (M. Myers, L. Rui).

Examples of some of this work include J. Williams' demonstration of the importance of mTOR in mediating the effect of dietary protein to regulate pancreatic acinar cell function, L. Samuelson's studies of Huntington related protein in gastric mucosa differentiation and apoptosis, D. Gumucio's studies of the role of Hedgehog signaling in defining the gastric duodenal junction. Other work is more related to specific function as for example R. Kaufman's studies of signaling from the endoplasmic reticulum to protect cells from ER stress which is now being applied to studies of pancreatic acinar and beta cells as well as hepatocytes and E. Stuenkel's study of the role of SNARE proteins in the mediation of exocytosis which is also being applied to pancreatic acinar and beta cells.

3. Pancreatic secretions and growth in health and disease

The Peptide Center has a long established interest in pancreatic biology. The principal investigators in this area include Drs. Williams, Owyang, Simeone, Andrews, DiMagno, and Stevens. Dr. John Williams and colleagues recently demonstrated that Rap1, a member of the Ras super family of small GTP-binding proteins, localized on pancreatic zymogen granules, plays an important role in pancreatic amylase secretion. Rap1 activation not only mediates the cyclic AMP-evoked response via Epac1, but is also involved in CCK and carbachol induced amylase release with the action most likely mediated by Ca/DAG-GEF III (9). Dr. Phil Andrews, working in collaboration with Dr. Williams' laboratory, has started a proteomics approach to study the zymogen granule. These investigators performed global topology analyses of pancreatic zymogen granule membrane proteins and showed two distinct clusters according to their isobaric tag for relative and absolute quantification (iTRAQ) ratios for proteinase K-treated versus control zymogen granules. The low iTRAQ ratio cluster includes cytoplasm-oriented membrane and membrane-associated proteins including myosin-V, vesicle-associated membrane proteins, syntaxin and all the Rap proteins. The second cluster having unchanged ratios includes predominantly luminal proteins. This study provides a firm foundation for developing a higher order architecture model of the zymogen granule membrane and for future functional studies of individual zymogen granule membrane proteins (10).

In collaboration with Dr. Steve Ernst, Dr. Williams' laboratory has also contributed significantly to the understanding of molecular mechanisms regulating pancreatic growth. They showed that CCK induces a spectrum of early response gene expression

and AP-1 binding in the pancreas. Since early response genes often couple short-term signals with long-term responses, these signals are likely to be important in mediating pancreatic growth (11). Dr. Williams also demonstrated that in vitro and in vivo CCK stimulates pancreatic growth through activation of the phosphatase calcineurin-NFAT signaling. This cellular mechanism appears to be critical for CCK to induce acini growth (12). In a separate but related study, Dr. Williams further demonstrated that CCK-induced pancreatic growth is not limited by acini cell mytogenic capacity but is due, at least in part, to inhibition of pro-mytogenic Akt signaling. This observation has important physiological and clinical implications in pancreatic dysfunction induced by protein malnutrition (13). In a mouse model of protein deficiency, it was demonstrated that dietary protein modulates pancreatic growth but not digestive enzyme synthesis via CCK-independent activation of the mTOR pathway (14-15).

Drs. Xuequn Chen and Steve Ernst in collaboration with Dr. Craig Logsdon, developed a new construct for mutant trypsinogen which is only activated intracellularly by the endogenous protease PACE. To demonstrate that localization of active trypsin determines its effects on pancreatic acini cells, these investigators showed that intracellular trypsin has no significant effect on the activity of the proinflammatory transcription factor NF- κ B. On the other hand, extracellular trypsin causes cell damage and dramatically increased NF- κ B activity. This observation significantly improves our understanding of the role of active trypsin in pancreatitis and its associated inflammatory response (16).

Cystic fibrosis transmembrane regulator (CFTR) gene mutations are associated with pancreatic insufficiency and pancreatitis. To understand the cellular mechanism responsible for this association, Drs. DiMagno and Owyang performed in vivo and in vitro studies using a CFTR (-/-) mouse model and showed that a baseline proinflammatory state and an antiapoptotic phenotype occur in this group of mutant mice and this may sensitize the animal to developing more severe acute pancreatitis with an exuberant pancreatic inflammatory response. These mice also have reduced pancreatic secretion in response to CCK stimulation and decreased pancreatic digestive enzyme protein mRNA levels suggesting mild pancreatic insufficiency. These observations may explain the susceptibility to pancreatitis in people with classic and non-classic cystic fibrosis (17).

In a separate study, Drs. DiMagno, Anderson and Owyang showed that the insulin sensitizing PPAR-gamma agonists ameliorate inflammation and fibrosis in a chronic pancreatitis rat model. They further demonstrated in a model of insulin resistance (endothelial NO syntase gene deleted mice) there is a 50% reduction in pancreatic exocrine secretion evoked by CCK and that treatment with PPAR-gamma agonists, pioglitazone restores insulin sensitivity and exocrine pancreatic secretion. This observation may have important clinical relevance to human chronic pancreatitis because the major risk factors are alcohol and smoking, and both uncouple eNOS from NO production (18). These investigators have now begun a clinical trial, supported by NIH (R21) to test the hypothesis that pioglitazone, by improving endocrine dysfunction, will improve pancreatic exocrine function and structure and reduce pain in chronic pancreatitis patients.

4. Physiology and pathophysiology of gastric parietal cell

Since the inception of the Center, gastric mucosal physiology has been a major research focus among many Center investigators. In a recent study, Dr. Linda Samuelson conducted Affymetrix microarray analysis to study transcriptional profiling of gastrin-regulated genes in the stomach. Her study showed that a number of inflammatory response genes are induced in gastrin deficient mice and normalize when acid is present in the stomach, suggesting these genes are increased in response to low gastric acid. In addition, a number of parietal cell transcripts are also downregulated in the gastric-deficient mice. They are restored in the presence of acid as well suggesting that parietal cell changes are also associated with hypochlorhydria. In contrast, the histamine-secreting enterochromaffin-like (ECL) cell genes that are markedly downregulated in gastrin deficient mice continue to be reduced in the presence of acid. These observations suggest that gastrin coordinately regulates a number of ECL cell genes, including several involved in histamine synthesis and secretion (19).

Drs. Andrea Todisco and Linda Samuelson have independently identified 2 important molecules, bone morphogenetic protein-4 (BMP-4) and Huntingtin interacting protein 1 related (Hip1r) as important regulators of parietal cell morphology and secretion. Dr. Todisco showed that BMP-4, a peptide synthesized by mesenchymal cells in the stomach plays an important role in parietal cell maturation and differentiation. This peptide stimulates Smad1 phosphorylation and induces H⁺/K⁺-ATPase gene expression. It also enhances histamine stimulated C¹⁴ aminopyrine uptake. Morphologically, BMP-4 promotes the induction and maintenance of a differentiated parietal cell phenotype (20).

In a separate study, Dr. Samuelson showed that Hip1r, an F-actin- and clathrin-binding protein, is expressed in gastric parietal cells. In Hip1r deficient mice, loss of tubulo-vesicle occurs in the parietal cells resulting in an enhanced activation and acid trapping. Electromicroscopy shows extensive apoptotic parietal cell death and glandular hypertrophy with cellular transformation. Taken together, these observations suggest that Hip1r plays an important role in gastric physiology, mucosa architecture and secretory membrane dynamics in parietal cells (21).

Dr. Juanita Merchant showed that gastrin deficient mice display gastric atrophy and metaplasia prior to progression to distal, intestinal-type gastric cancer. This appears to be regulated by abnormal regulation of sonic hedgehog (Shh). In canine parietal cells, gastrin stimulates Shh gene expression and acid-dependent processing of the 45-kDa Shh precursor to the 19-kDa secreted peptide. This cleavage is blocked by a proton pump inhibitor and is mediated by the acid-activated protease pepsin A. This observation suggests that processing of Shh in normal stomach is gastrin regulated, acid-dependent, and mediated by the aspartic protease pepsin A. Loss of Shh processing may cause parietal cell atrophy, a known preneoplastic lesion in the stomach (22).

5. Vagal physiology and digestive functions

Studies from Drs. Li and Owyang's laboratories showed that vagal afferent pathways represent the primary targets by which peptide hormones and circulating nutrients modulate digestive functions. Combining single nodose ganglia neuron recording, immunocytochemistry and in vivo motility and secretion studies, Drs. Li and Owyang

demonstrated that CCK acts mainly on the vagal nodose ganglia to mediate pancreatic enzyme secretion and induce satiety. Recently Dr. Owyang's laboratory further characterized the neurocircuits in the dorsal motor nucleus of the vagus (DMV) and showed that there is a high degree of spatial organization of the neurons synapsing with intragastric cholinergic and nitric oxide/VIP neurons in the rat. Depending on the location of the stimulation (rostral or caudal), L-glutamate may activate DMV neurons to mediate gastric contractions and relaxation. Cholinergic neurons in the gastric corpus mainly respond to L-glutamate stimulation of neurons in the rostral part of the DMV; both nitrenergic and VIPergic neurons are responsive to L-glutamate stimulation in the caudal part of the DMV producing relaxation. This spatial organization of the DMV cholinergic neurons provides the structural basis for differential regulation of the DMV neurons projecting to the stomach. Different groups of glutamate-containing neurons from the brain stem or higher centers projecting to the DMV may regulate gastric contraction or relaxation depending on whether they synapse with cholinergic neurons in the rostral or caudal part of the DMV (23).

Nutrients in the circulation play an important role in the regulation of motility and eating behavior. Clinically, it is well known that hyperglycemia has a profound inhibitory effect on gastric motility. However, little is known about the site and mechanism that sense alteration in blood glucose levels. In a recent study, Dr. Owyang and colleagues demonstrated that hyperglycemia inhibits gastric motility via a capsaicin-sensitive vagal afferent pathway originating from the gastric duodenal mucosa. Hyperglycemia stimulates vagal afferents, which, in turn, activate vagal efferent cholinergic pathways synapsing with intragastric nitric oxide- and VIP-containing neurons to mediate gastric relaxation (24). These findings have obvious clinical importance. It suggests that hyperglycemia alone, in the absence of underlying neuropathy or myopathy, can alter gastric motor function. This may explain the common clinical observation that diabetic patients with stable motor defects often exhibit wide day-to-day variations in the severity of their symptoms depending on blood glucose control.

To further demonstrate that glucose responsive neurons are present in the nodose ganglia, Dr. Gintautas Grabauskas, a Center Associate Investigator working in collaboration with Dr. Owyang, combined patch-clamp recording, single cell RT-PCR, immunocytochemistry and signal transduction studies, and demonstrated that 26% of the nodose neurons are glucose-excited whereas 16% are inhibited by high glucose. These specialized neurons utilize the product of intracellular glucose metabolism to regulate the activities and transmitter release. In the glucose-excited neurons, uptake and metabolism of glucose lead to closure of the K_{ATP} channels, triggering a membrane depolarization. In contrast, in the glucose-inhibited neurons, high glucose triggers cyclic AMP synthesis which in turn acts via Epac-PLC pathway to activate a 2 pore K^+ channel, TRESK resulting in hyperpolarization. This is the first demonstration of the existence of a subpopulation for glucose-excited and glucose-inhibited neurons in the nodose ganglia. Understanding peripheral glucose sensing as it relates to digestive functions may provide important information in the treatment of diabetic patients with gastrointestinal complications (25).

Works in the laboratories of Drs. Li and Owyang have also contributed to the understanding of vagal functions in disease states. For example, recent clinical studies suggest that low frequency vagal stimulation may provide relief of nausea and dyspeptic symptoms in patients with diabetic gastroparesis. Using a diabetic rat model, Dr. Li's laboratory showed that these animals have a lower pain threshold and enhanced

activation of anterior cingulate cortex neuronal firing in response to colorectal distension. Stimulation of vagal afferent A-fibers but not C-fibers reduce visceral pain and this requires the activation of the periaqueductal gray-descending pain-inhibitory pathways which reduce spinal nociceptive transmission. This provides the functional basis of using low frequency vagal electrical stimulation and may potentially be useful in the treatment of chronic visceral pain (26).

Anti-cancer chemotherapy often induces emesis. This action has been attributed to the release of serotonin (5HT) from intestinal EC cells which act on vagal 5HT₃ receptors. Recently NK-1 receptor antagonist has shown some efficacy in preventing delayed emesis following chemotherapy, Dr. Li and his colleagues demonstrated that anti-cancer therapy with cisplatin causes plastic changes in the nodose ganglia characterized by upregulation of NK-1 receptor in the vagal nodose ganglia. This is mediated by the enhanced release of 5HT from intestinal EC cells resulting in activation of NK-1 receptors. The interaction of substance P and serotonin in vagal afferent neurons appears to be responsible for the delayed emesis observed in anti-cancer therapy. This finding supports the combined use of 5HT₃ and NK-1 receptor antagonists to deliver a more effective control of post chemotherapy emesis.

6. GI Motility Studies

The GI Division at University of Michigan has a strong group of GI motility investigators. They include Drs. Hasler, Wiley, Chey, Saad, Hoogerwerf, Owyang, as well as Khalil Bitar. Some recent advances are highlighted as follows.

Dr. Shiyi Zhou, an Associate Investigator, supported by the American Diabetic Association and working in conjunction with Dr. Owyang, investigated the mechanisms responsible for the selective loss of interstitial cell of Cajal in diabetic stomach. His study demonstrated that mosidomine, a nitric oxide (NO) donor, prevents damage of diabetic rat gastric intramuscular interstitial cell of Cajal (IM-ICC). His studies showed that nitric oxide regulates the frequency of ICC currents and that both nitric oxide synthase (NOS) containing neurons and ICC are damaged in the myenteric plexus of 6 week diabetic rats. On the other hand, diabetic rats did not have reduction in acetylcholine containing neurons. Treating diabetic rats with mosidomine prevented the loss of ICC but did not prevent apoptotic loss of NOS containing neurons. These findings suggest that selective impairment of nitrergic function leads to gastric IM-ICC damage in diabetic rats. NO-donors may prevent gastric IM-ICC damage, thus reducing gastric dysmotility in diabetics. These findings may have therapeutic implications in the treatment of diabetic gastroparesis.

It is well known that hyperglycemia evokes tachygastria via the prostaglandin (PG) pathway in experimental animals and humans. To investigate the mechanism responsible for this problem, Dr. Owyang and colleagues performed mapping of gastric electrical activities using Teflon coated wires anchored to the gastric corpus and antrum. Their study showed that both hyperglycemia and PGE₂ inhibit native pacemaker activities in the corpus but activate atopic electrical activities in the antrum. This is mediated by differential distribution of EP₂ and EP₃ receptors in these regions of the stomach. Activations of EP₂ receptor inhibit gastric electrical activities in the corpus whereas stimulation of EP₃ receptors stimulates atopic pacemaker activities in the antrum. In this manner, hyperglycemia through the differential actions of prostaglandin in the corpus and antrum evoked tachygastria in rats. These findings offer insight into the

myenteric disturbances in diabetic patients and suggest possible therapeutic roles for inhibitors of prostaglandin receptor subtypes for gastric dysrhythmias in this condition.

The Center has played a significant role in the successful development of the colonic clock gene project of Dr. Sandra Hoogerwerf. The results were published in *Gastroenterology* (27-28). She was the first to demonstrate the significance of clock gene expression in the murine GI tract. Her study showed clock gene immunoreactivity was observed in the myenteric plexus and epithelium crypt cells. Time feeding shifted clock gene expression at the RNA and protein level but did not affect clock gene expression in the central clock (CNS). Vagotomy did not alter gastric clock gene expression. The presence of clock genes in myenteric plexus and epithelial cells suggests a role for clock genes in circadian coordination of GI functions such as motility, cell proliferation and migration. To investigate the roles of clock genes on the biological rhythms in colonic motility, Dr. Hoogerwerf developed a novel telemetric method of measuring colonic motility in conscious, free moving mice. This novel method allowed acquisition of colonic motility data over a period of days. Her studies demonstrated that colonic motility showed diurnal variations, with increasing colonic motility pressure at the onset of dark cycle. This coincides with the time that nocturnal animals consume most of their food. It is interesting to note that a subset of clock genes in the mouse colon which are crucial for the regulation of colonic motility such as nitric oxide synthase and vasointestinal peptides, also showed rhythmic expression. Current ongoing studies attempt to correlate the motility changes with the circadian rhythms of the clock genes. This set of observations has important physiological and pathophysiological implications. Human colonic motility studies indicate that most individuals show maximal increase in motility upon awakening and minimal activity during the night, a pattern similar to that observed in mice as reported by Dr. Hoogerwerf. Disruptions of our daily rhythm such as occur with shift work or different time zone travel has been associated with GI symptoms such as abdominal bloating and change of bowel habits. The pathogenesis of these symptoms may be related to the malfunctioning of our peripheral clock genes, resulting in a disturbance in the biological rhythms of colonic motility. This is the major thesis of Dr. Hoogerwerf's research.

The Peptide Center provides important technical support to Dr. Khalil Bitar to develop a bioengineered, innervated, internal, anal sphincter (IAS) ring which may help patients with severe fecal incontinence. Dr. Bitar and his team were able to successfully implant bioengineered rings which were constructed from IAS smooth muscle cells and neuronal precursor cells. The innervated constructs showed normal response to stimulatory and inhibitory neurotransmitters. Similar results were obtained from constructs bioengineered using human IAS smooth muscle cells co-cultured with IM-FEN cells. The implanted rings became vascularized and survived in animals without any signs of rejection for up to one month. This is the first demonstration of physiologically functional bioengineered innervated smooth muscle constructs and may one day lead to an innovative approach to the treatment of fecal incontinence (29).

7. Neural peptides and regulation of visceral pain

The molecular mechanisms responsible for the pathophysiology of visceral pain remain largely unknown. Progress in visceral pain research has been plagued historically by inadequate quantitative methodological approaches. During the last decade, Center investigators have taken advantage of recent advances in molecular biology, electrophysiology, imaging technologies and behavior research and made

significant advances in our understanding of the pathophysiology responsible for visceral pain in a number of clinical conditions. Major pain research groups in the Center include the laboratories of Drs. Wiley, Isom, Morrow and Owyang. There are two main research themes: (a) pathophysiology of painful diabetic neuropathy and (b) role of neuropeptides and their receptors in the mediation of visceral hypersensitivity. These research groups have received support from all of the Peptide Center Cores (Molecular, Biology, Cell Biology, Proteomics and In Vivo Studies). The following highlights some of the new findings which enhance our understanding of these complex clinical disorders.

Dr. John Wiley's laboratory continues to search for mechanisms which contribute to sensory neuropathy in diabetes. Previously these investigators reported that sera from patients with type 2 diabetes and neuropathy induce autophagy in human neuroblastoma (SH-5Y5Y) cells. More recently, they showed that enriched immunoglobulin fractions from this group of patients activate the Fas cascade and induce autophagy (30). Similar observations were made from sera of patients with idiopathic chronic intestinal pseudoobstruction (31) suggesting that this phenomenon is likely to be a secondary phenomenon following initial nerve injuries in diabetes.

Diabetes also induces extensive changes in receptor expressions and signal transduction mechanisms in sensory DRG neurons. Dr. Wiley's laboratory previously demonstrated that calcium entry is increased in diabetic neurons and associated with the activation of program cell death. This appears to be due to altered inhibitory G protein functions. Diabetic DRG neurons also showed increased expression and function of the transient receptor potential vanilloid 1 (TRPV1). These neurons display increased oxidative stress and activation of cell injury markers (caspase 3) compared with healthy controls. Treatment with capsazepine, a competitive TRPV1 antagonist, markedly reduces these abnormalities in vitro and prevents activation of cell injury in large DRG neurons of diabetic rats in vivo (32). Hence abnormal receptors and signals transduction may contribute to preferential neuronal stress in DRG neurons in diabetic sensory neuropathy.

Visceral hypersensitivity is frequently observed in diabetic patients with sensory neuropathy. It may be responsible for abdominal pain and rectal urgency in this group of patients. The molecular mechanisms responsible for this abnormality are largely unknown. Dr. Isom's laboratory is focused on the molecular biology regulating the subunits of sodium channels in sensory DRG neurons. Her recent studies showed that voltage-gated sodium channel (Na(V)1) beta subunits modulate channel gating, assembly and cell surface expression in CNS neurons. In collaboration with Dr. John Wiley in a study supported by the Center as a pilot feasibility study, these investigators investigated whether alteration of the beta subunits contributes to the visceral hypersensitivity in clinical neuropathy. They showed that beta 2 expression increases in sensory neurons after nerve injury such as diabetic neuropathy, and development of mechanical allodynia in the spared nerve injury model is attenuated in beta 2-null mice. These investigators reported that the small DRG neurons isolated from beta 2 (-/-) mice showed significant decreases in TTX-S Na current compared with beta 2 (+/+) neurons. The TTX-S but not TTX-R current activation and inactivation kinetics in these cells are slower in beta (-/-) mice compared with controls. These changes are accompanied by reductions in transcripts and protein levels of Na(V) channels. Thus it appears that during nerve injury such as diabetic neuropathy, beta 2 subunits modulate TTX-S Na(V) channel mRNA and protein expression, resulting in increased TTX-S(Na) in small fast DRG neuron (33). Targeting beta subunits of voltage-gated sodium channel in the DRG

neurons may represent a potential approach for the treatment of painful diabetic sensory neuropathy.

In a separate but related study, Dr. Owyang's laboratory performed patch-clamp studies on isolated sacral DRG neurons from streptozocin induced diabetic rats. They found major alterations in electrophysiological properties in a subgroup (type II) of DRG neurons which become more excitable. They are characterized by a marked increase in membrane resistance and a significant reduction in I_A current amplitude. This appears to be mediated by an increase of intracellular Ca^{2+} and activation of MAPK cascades. Administration of Ca^{2+} channel blockers or MAPK inhibitor PD98059 restores the I_A current and normalizes the excitability of the diabetic DRG neurons in vitro. Parallel in vivo studies showed that intrathecal administration of the MAPK inhibitor PD98059 corrects the rectal hypersensitivity in response to rectal balloon distension in diabetic rats. Studies to demonstrate that activation of the MAPK cascades results in internalization of the K^+ channels responsible for I_A currents are ongoing.

Visceral hypersensitivity in diabetes is not only restricted to abnormalities in the DRG neurons. Dr. Thomas Morrow's laboratory in collaboration with Dr. Wiley showed that 4 weeks after streptozocin, diabetic rats exhibit behaviors indicative of neuropathic pain. Brain imaging studies using (99 M) Tc0HMPAO reveal significant increases in the activation of brain regions involved in pain processing. These regions include secondary somatosensory cortex, ventrobasal thalamic nuclei and the bilateral amygdala. In contrast, the activation of habenular nuclei and the midbrain periaqueductal gray are markedly decreased. These findings suggest that pain in diabetic neuropathy may be due in part to hyperactivity in somatosensory structures coupled with a concurrent deactivation of structure mediating antinociception (34). Drs. Morrow and Wiley are planning to conduct CNS PET scan studies in diabetic patients with painful neuropathy to corroborate these findings.

8. Human studies

The In Vivo core of the Center plays an important role in promoting human research and facilitates the translation of basic discoveries to clinical application. A major accomplishment of the Center during the last several years was the development of a blood and tissue bank for inflammatory bowel disease, colon and esophageal cancer, pancreatic cancer, and visceral neuropathy. In addition, the Center also has recruited a number of clinical study coordinators to facilitate clinical and translational studies performed by Center investigators. This new facility has greatly promoted clinical studies by providing blood and tissue samples for in vitro testing. A number of examples are cited below as samples achievements by our investigators.

Under the leadership of Drs. Ellen Zimmermann and Peter Higgins, a database is established for IBD patients which contains detailed clinical information on the phenotype of patients with Crohn's disease and Ulcerative Colitis. In a study supported by NIH and led by Drs. Zimmermann and Nunez (R01 DK067628; R01 DK073992), blood is processed for genotyping for common NOD2 and other relevant mutations. The study aims to determine if NOD2 mutations are associated with strictures or other bowel findings identified by CT enterography. Three common NOD2 mutations including SNP-8, SNP-12, and SNP-13 were investigated. During the last year, 91 patients were genotyped and showed that patients with SNP-13 mutation have a 5.1 fold increase in risk of strictures on CT enterography. On the other hand, SNP-8 or SNP-12 mutations

alone have no significant stricture risk. Logistic regression demonstrated a dose effect with each additional mutation adding a 4.3-fold increase odds of stricture ($P < 0.05$). This data supports the association between NOD2 mutations and small intestinal strictures and may be used as biomarkers to predict the natural histories of patients with Crohn's disease. This may influence our approach in the clinical management of this group of difficult disorders.

The Center's clinical core also provides logistic support to Drs. Joel Rubenstein and John Kao for their search for biomarkers for Barrett's esophagus (K23 DK59111; R03 DK075842). Adiponectin is a peptide secreted by adipose tissue, whose blood levels are inversely correlated with obesity and lower in men than women. It is involved in regulation of inflammation and may suppress carcinogenesis by a number of mechanisms (35). Specific receptors for adiponectin (AdipoR1 and AdipoR2) are found in the esophageal mucosa and adiponectin induces apoptosis in a cell line of esophageal adenocarcinoma (36). To test the hypothesis that circulating levels of adiponectin and its multimers are associated with risk of Barrett's esophagus, blood samples were collected from 112 cases of Barrett's esophagus and 199 GERD controls. It was demonstrated that high levels of low molecular weight adiponectin are associated with a decreased risk of Barrett's esophagus (3rd tertile vs. 1st tertile, aOR=0.33, 95% CI=0.16, 0.69) and a high low molecular weight/total ratio appears particularly inversely associated with Barrett's esophagus. This is the first demonstration that there is a strong inverse relationship between circulating levels of low molecular weight adiponectin and the presence of Barrett's esophagus among patients with GERD (37). If confirmed in a larger number of patients, this finding has implication, both on models of pathogenesis of Barrett's esophagus as well as for potential use as a biomarker of disease. In vitro studies are underway to understand if there is a mechanism whereby adiponectin may promote Barrett's esophagus and metaplasia.

Pancreatic cancer is a formidable disease and early detection biomarkers are needed to make inroads into improving the clinical outcomes in this group of patients. Recently, Drs. Diane Simeone and Michelle Anderson have identified 4 potential glycoprotein markers. To investigate whether these molecules are detected in blood, serum samples were collected from 89 normal controls, 35 chronic pancreatitis, 37 diabetic samples and 22 pancreatic cancer patients. Glycoproteins were evaluated on the microarray in situ by on-plate digestion and direct analysis MALDI QIT-TOF mass spectroscopy. These investigators found that it was possible to discriminate cancer from other disease groups and normal samples with high sensitivity and specificity where the response of Alpha-1-Beta glycoprotein to lectin SNA increased by 69% in the cancer sample compared to other non cancer groups. This data suggests that differential glycosylation patterns detected on high-throughput lectin glycol-antibody microarrays are promising biomarker approach for the early detection of pancreatic cancer (38).

In a clinical study Dr. John Wiley collaborated with Dr. Roberto DeGiorgio to investigate whether sera from a subpopulation of patients with chronic intestinal pseudoobstruction contained an antibody that might activate autophagy in a cultured human neuroblastoma cell line. In total, 25 patients with established neurogenic chronic intestinal pseudoobstruction were investigated and circulating antineuronal antibodies to enteric neurons were found in 6 (24%) patients. These investigators showed that exposure of the neuroblastoma cells (SH-Sy5Y) to sera from patients with chronic intestinal pseudoobstruction containing antineuronal antibodies increased the binding of autoimmunoglobulin to the surface of the SH-Sy5Y cells and increased the formation of

autophagosomes showing colocalization with mitochondria and Fas-activated death domain compared with control sera. Pretreatment of sera with either protein L agarose beads or soluble Fas receptor chimera prevented the stimulation of autophagy. This study provides novel evidence that antineuronal antibodies may contribute to neuronal dysfunction observed in a subset of patients with neurogenic chronic intestinal pseudoobstruction via autoantibody-mediated activations of autophagy involving the Fas receptor complex (31).

The human motility research group received invaluable support from the Center for their clinical motility investigations. In collaboration with the Department of Bioengineering, Drs. William Hasler and Chung Owyang developed an apparatus for gas collection to be used in conjunction with a barostat to evaluate the patterns of gas transit in the small bowel. Using this novel technology, the investigators showed that caloric meals promote bolus gas transit in healthy humans, whereas non caloric liquids have no effect. Solids stimulate early postprandial gas dynamic to the same extent as liquid meals of similar caloric content. Thus it appears that modulatory effects of meals on intestinal gas transit depend on their caloric content but not their consistency (39). In a companion study, Drs. Hasler and Owyang demonstrated that consumption of a high fiber diet retards intestinal gas transit by decreasing bolus propulsion to the rectum. Hence, in addition to increasing gas production by colonic flora, fiber ingestion may elicit gaseous symptoms by promoting gas retention (40). Similar techniques will be performed to investigate the pathophysiology of gas and bloating in patients with IBS.

With the support of the In Vivo Core, Drs. William Chey and William Hasler participated in a multicenter trial to investigate the clinical usefulness of a new wireless pH and pressure recording capsule (SmartPill®). In a study involving 78 constipated (Rome II) and 87 healthy subjects, they showed that the diagnostic accuracy of the SmartPill colonic transit time to predict constipation was 0.75 with a specificity of 0.95. These are comparable with the radiopaque marker studies. This study also revealed unrecognized gender differences and upper gut dysfunctions in constipation. This new technology shows promises as a new method to evaluate gastrointestinal motility (41).

With better electrode design and improved software, Center investigators Drs. Wiley, Hasler and Owyang performed mucosa mapping of electrical activity in human subjects. Bipolar electrodes were fixed 10.5, 6, and 2 cm from the pylorus during endoscopy in 10 healthy subjects. Glucose clamp studies were performed to study the effects of acute hyperglycemia on slow waves in different regions of the stomach. Their studies demonstrated that acute hyperglycemia elicits isolated tachygastrias and uncoupling of normal slow waves that are most prominent in the distal stomach. In contrast, hyperglycemia has a limited effect on power gradients and does not disrupt gradients in power variability or the conduction velocity of normally coupled slow wave. These findings provide insight into myoelectric disturbances which may underly gastric functional abnormalities of diabetic gastropathy and provide a foundation for studying slow wave conduction defects in this condition (42).

C. MAJOR RESEARCH THEMES

The Peptide Center continues to highlight three thematic areas that have led to exceptional cross disciplinary collaboration amongst the numerous investigators

affiliated with the Center. The themes that reflect common research interests are 1) Neurobiology in Appetite Control, Metabolism and Visceral Pain; 2) Molecular and Cellular Mechanisms of Inflammation; and 3) Cell Growth, Differentiation and Programmed Cell Death. Collectively these themes include many of the poorly understood areas in which peptides regulate gastrointestinal function and disease. The thematic organization has proved to be a successful nidus for collaboration, core utilization as well as intellectual exchange. The Peptide Center's cultivation and stimulation of novel research disciplines is reflected in the productivity and ongoing enthusiasm of over seventy investigators who continue to invigorate the center.

During the last 5 years, much has been achieved in these 3 areas of research which will remain the major research focus of the Center even as they continue to dynamically evolve. Within the first theme, appetite control has expanded to include regulation of metabolism and energy balance. For visceral pain, the future focus will include understanding the molecular mechanisms responsible for visceral hypersensitivity involving the dorsal root ganglia and anterior cingulate cortex. Within the second theme, the arena of inflammation has added a new focus in microbiome as it relates to intestinal inflammation, in addition to plans to study the role of peptides in inflammation in models of steatohepatitis. To enhance translational research in our third theme involving cell growth differentiation and programmed cell death, we have included a new program on the use of confocal microendoscopy for early detection of GI malignancy. Investigators in each of these research areas will utilize a broad range of Core resources provided by the Center to enhance cross disciplinary collaboration and facilitate a collective research milieu.

Thematic Executive Summaries

Theme 1: Neurobiology in Appetite Control, Metabolism and Visceral Pain

Obesity is an epidemic worldwide health issue associated with coronary disease, hypertension, diabetes, fatty liver disease and the metabolic syndrome. The peptide center has a large group of investigators dedicated to elucidating the mechanisms by which neuropeptides regulate satiety and metabolism. Dr. Owyang's group is collaborating with other center investigators to study cholecystokinin and leptin molecular mechanisms of action in the pancreas and nodose ganglion. Dr. Myers' group is studying leptin and leptin receptor signaling using molecular probes, cell culture and genetically modified mouse models. Dr. Malcolm Low's group is elucidating the role of the hypothalamus in integrating environmental and interoceptive sensory information for energy balance and neuroendocrine homeostasis. Dr. Liang-you Rui is also studying hypothalamic pathophysiology with regard to satiety and energy balance. Dr. Jiandie Lin is studying the rhythmic regulation of PGC-1 alpha which regulates energy metabolism in the liver and skeletal muscle, which is leading to a better understanding of how clock genes regulate energy metabolism.

Visceral hypersensitivity is an important unifying theme for clinical disorders which affect millions of Americans including non-ulcer dyspepsia, non-cardiac chest pain, and painful irritable bowel syndrome. In a large collaborative effort, Drs. Owyang, Wiley, Li, and Isom continue their efforts to elucidate the role of peptides in molecular pathways to visceral hypersensitivity and establish their correlation with important clinical disease entities. The investigators are applying gene targeting, electrophysiology, cell and molecular biology along with emerging selective pharmacologic neuronal modulation

with siRNA technology to study these important pathways. Taken together, these efforts will continue to increase our knowledge of the molecular etiologies of visceral hypersensitivity so we may better serve people afflicted with these dreaded conditions.

Theme 2: Molecular and Cellular Mechanisms and Inflammation

The role of peptides in inflammation is being pursued as it relates to two major clinical entities in gastroenterology, *H. pylori* gastritis and inflammatory bowel disease. Center investigators are applying powerful emerging molecular genetic techniques to characterize microbiomes in the context of hypothesis driven research on the role of peptides in inflammation. Seminal investigations include the world renowned work of Dr. Steven Kunkel on cytokine and chemokine biology. Dr. Kunkel continues to mentor and collaborate with a myriad of center investigators including Drs. Zimmermann, Higgins and Kao. Dr. Deborah Gumucio's lab continues to elucidate the role of peptides to mediate inflammation, particularly in the function of gut epithelial cells. Dr. Gumucio collaborates closely with Peptide Center investigators, including Drs. Merchant and Samuelson. The center members are all quite proud that Dr. Merchant's accomplishments led to her induction into the Institute of Medicine this year. Dr. Merchant's independent career has largely been conducted in the collaborative intellectual environment supported by the Peptide Center and its cores, so her achieving one of the highest recognitions afforded a physician scientist in our country reflects positively on her research environment as well.

Gabriel Nunez's laboratory is acclaimed for studying the role of peptides in the regulation of Toll-like receptors and nod-like receptors. Dr. Nunez has trained and collaborated with many of the center's more junior investigators, including Dr. Grace Chen. Dr. Kao is studying the role of peptides in modulating inflammation through professional antigen presenting dendritic cells. He is particularly interested in dendritic cell peptide mechanisms that may be exploited to down regulate inflammation in health and disease. Other center investigators studying the role of peptides in inflammation include Drs. Merchant, Shah, Higgins, Zimmermann, Huffnagle and Young. The microbiome core led by the later two investigators is providing an exceptional novel set of tools to center investigators who study inflammation.

We are particularly excited to have Dr. Bishr Omary join the Molecular and Cellular Mechanisms and Inflammation theme with his research on hepatic steatosis and peptide mediators of inflammation. His work includes collaborations with two other center investigators, Dr. Randal Kaufman and Yatnik Shah, who together are elucidating novel mechanisms of inflammation in fatty liver disease. This work includes studying keratin intermediate filaments (IF), ER-Stress-Mediated Suppression of Transcriptional Master Regulators, and inflammatory signaling in the setting of intrahepatic hypoxia.

Theme 3: Cell Growth, Differentiation and Programmed Cell Death

Drs. Merchant, Samuelson, Gumucio, and Dlugosz continue to study the role of peptides in regulating cellular growth and development pathways, which are often misregulated in gastrointestinal disease. They are studying the role of inflammatory mediators and hedgehog signaling pathways in regulating health and disease. In

addition to their close collaboration with each other, they also work closely with other center members such as Dr. Todisco whose work on the peptide BMP-4 elucidates its mechanism in induction and maintenance of parietal cell phenotype.

Dr. Sean Morrison, another distinguished Peptide Center investigator, is studying the mechanisms of neural stem cell renewal, differentiation and aging. Dr. Hisashi Umemori is studying mechanisms of synaptic development in the enteric nervous system. With regard to cancer stem cells, Dr. Diane Simeone is identifying cancer stem cells in gastrointestinal tumors using distinct peptide cell surface markers. Dr. Collin Duckett focuses on peptides derived from the inhibitor of apoptosis (IAP) gene family. Dr. Kaufman is studying mechanisms of intracellular signaling on protein synthesis and fidelity of protein structure.

One new direction which continues to invigorate center members is the use of In vivo imaging of gene and protein expression in a translational component of this Peptide Center theme which is led by Dr. Thomas Wang. Dr. Wang, along with his collaborators in the engineering school, is developing a probe for detecting colon cancer using data obtained by screening phage display peptide libraries. In collaboration with other center investigators, including Drs. Eric Fearon and Chung Owyang, Dr. Wang is developing radio-labelled peptides to localize dysplasia on cross sectional imaging, as well as with confocal microscopy and to develop a scanning fibroendoscope.

The following paragraphs provide more details on the accomplishments of each of the laboratories with key findings demonstrative of the research work accomplished in the three thematic arenas. They are mainly provided for readers who wish to have more elaborate information about the research work and their physiological and/or clinical implications.

1. Neurobiology in Appetite Control, Metabolism and Visceral Pain

(a). Regulation of appetite and metabolism

Obesity is a major health problem worldwide. It may lead to myriad of serious health problems including heart disease, hypertension, diabetes and metabolic syndrome. Defective appetite regulation and/or abnormal metabolism may result in energy imbalance and obesity. Therefore, understanding the complex mechanism regulating eating behavior and metabolism has important clinical significance. Since its inception, the Center has a large group of investigators whose research is focused on the role of neuropeptides in the regulation of satiety and metabolism. During the last funding period, there has been some significant changes in the key investigators in this research arena. Dr. Ira Gantz, whose major research focus is on the role of the melanocortin system in appetite regulation, has left the University of Michigan in 2006. Dr. Stan Watson and Dr. Huda Akil, founding members of the Peptide Center and key investigators on the role of serotonergic, dopaminergic, and opiate pathways in the CNS, have shifted their research focus to affective disorders and thus are playing only a peripheral role in the research of appetite and its regulation. However the Center is very fortunate to have recruited 5 prominent research scientists whose research focuses on investigating the mechanisms that regulate energy metabolism as it relates to the GI tract. These investigators include Drs. Martin Myers, Malcolm Low, Lian-you Rui, and Jiandie Lin. This team of researchers demonstrates the extraordinary talent on an emerging area related to eating behavior, energy balance and metabolism.

Cholecystokinin (CCK) and leptin are two important peptides involved in the regulation of short- and long-term eating behaviors. Dr. Owyang's laboratory has a long-established interest in the biology of CCK as it relates to pancreatic secretion and satiety. Their recent work clearly indicates CCK's actions in pancreatic secretion are mediated by high affinity CCK-A receptors whereas low affinity receptors regulate eating behavior. Electrophysiological and c-Fos immunoreactivity studies showed that low and high affinity CCK receptors are present in different nodose ganglia neurons and those co-express with leptin receptor are low affinity CCK-A receptors. Their recent studies indicate that the short-term satiety action of CCK is entirely dependent on leptin. In lean mice, leptin did not modify food intake for the first 4 hours post injection, but induced a 40% reduction in food intake during post injection hours 5-7. On the other hand, administration of CCK-8 reduced food intake by 33% in the first 3 hours, food intake was thereafter no different from leptin alone. Co-administration of leptin and CCK markedly enhanced CCK's inhibitory actions resulting in a 56% inhibition 3 hours post injection. In the ob/ob leptin deficient mice, CCK alone did not affect food intake. Co-administration of leptin-CCK reduced food intake similar to that observed in lean mice. It is interesting to note that CCK stimulates pancreatic protein secretion to a similar degree in lean and ob/ob mice suggesting that pancreatic action of CCK is not leptin-dependent. This study indicates that leptin not only serves as a regulator of long-term feeding behavior, by interacting with CCK, it also acts as a mediator of short-term satiety.

The synergistic interaction between CCK and leptin in the nodose ganglia is critical for CCK to exert its action to mediate short-term satiety. Subsequent studies in Dr. Owyang's laboratory demonstrated that this interaction is mediated by phosphorylation of STAT3, which in turn, activates the closure of potassium channel leading to membrane depolarization and neuronal firing in the nodose ganglia neurons. This involves the interaction between CCK/SRC cascades and the leptin/JAK/PI3/STAT3 signaling pathways. Studies to characterize this novel action of STAT3 on regulating potassium channel activity are underway. It is conceivable that malfunctioning of this signaling molecule may result in eating disorders.

Research in Dr. Martin Myers' lab focuses on the biology of leptin, which regulates physiological processes relevant to diabetes and the metabolic syndrome. Adipocytes secrete leptin to convey information about nutritional and metabolic status by activating the leptin receptor (LepRb) on certain neurons in the brain. These neurons regulate metabolism (including glycemic control) and endocrine function. Mutation of leptin or LepRb in rodents and humans results in profound diabetes, metabolic syndrome, and endocrine failure. Many LepRb-expressing neurons (including those in the hypothalamus and brainstem) also receive input from a variety of gut peptides. Dr. Myers' lab takes a broad-based approach to understanding leptin action by defining the mechanisms of cellular LepRb action, studying the neurons that express LepRb, and determining the roles of these molecular and neural mediators in leptin action in vivo.

Over the last few years, Dr. Myers' laboratory has made research strides in studying the mechanisms of LepRb signaling, as well as the roles of individual LepRb signals in the physiologic response to leptin, and continues to explore specifics about cellular mechanisms of leptin action. Current areas of focus include roles for Jak2, STAT5, and IRS proteins in leptin action. The Myers lab continues to study these mechanisms in cultured cells in addition to examining their physiologic roles in genetically modified mouse models.

While two well studied populations of LepRb-expressing neurons in the arcuate nucleus of the hypothalamus mediate important aspects of leptin action, these neurons only account for a fraction of leptin action; indeed they only comprise approximately 20% of all LepRb-expressing neurons in the brain. The Myers lab is therefore scrutinizing several other major populations of LepRb neurons in the brain; the function of these novel populations of LepRb-expressing neurons is likely to be relevant to diabetes and other aspects of the metabolic syndrome and endocrine function. Included in these studies is the examination of how a variety of gut-derived peptides modulate specific groups of LepRb-expressing neurons. In these studies, Dr. Myers is collaborating with the laboratories of Drs. Owyang and Merchant.

It is also clear that leptin controls the early development of at least some of the neural populations which are involved in the control of glucose homeostasis and metabolism. This developmental regulation may be involved in the programming of the predisposition to diabetes and the metabolic syndrome. Dr. Myers' lab is developing genetic models that will facilitate the interrogation of the mechanisms and consequences of this leptin-mediated neural programming. Much of this work has been and continues to be heavily supported by the Transgenic Animal Model Core and the Vector Core of the Center.

Dr. Malcolm Low's lab is focused on determining how the hypothalamus integrates environmental and interoceptive sensory information to maintain neuroendocrine homeostasis and energy balance. He is an internationally recognized expert in the generation and analysis of mutant mouse models and currently uses a combination of molecular genetic, endocrine, and behavioral approaches to characterize the physiological functions of neuropeptides and G-protein coupled receptors that are highly expressed in hypothalamic neural circuits, particularly proopiomelanocortin (POMC), MC4-receptor, enkephalin, dynorphin, mu-opioid receptor, corticotropin releasing hormone (CRH), somatostatin (SST), dopamine, and dopamine D2-like receptors.

POMC neurons play a critical role in the regulation of appetite and metabolism and dysfunction in their associated neural circuits can produce morbid obesity. In one project, Dr. Low and his colleagues analyze the modulatory role of glucocorticoids in the pathophysiological mechanisms underlying hyperphagia, altered meal patterns, and the metabolic syndrome exhibited by mutant mice with either constitutive or neural-specific deletions of the POMC gene. They combine novel retrograde and anterograde neural tracing techniques based on genetically expressed markers to define the afferent and efferent synaptic connections between hypothalamic POMC neurons and other brain regions. The role of phenotypically distinct subpopulations of POMC neurons in the regulation of energy homeostasis is being analyzed using diploid aggregation chimeras. Related to this project, they are also studying endogenous opioids and their interaction with sex steroids on the rewarding and motivational properties of food using operant behavioral paradigms.

In a second major project, Dr. Low used a combination of transgenic reporter expression and phylogenetic DNA footprinting to identify two discrete neuronal-specific enhancers in the *POMC* gene. Physiological and biochemical studies using mice with induced mutations in the enhancer DNA sequences will determine whether the two enhancer regions have redundant or unique functional roles in neuron-specific transcription of *POMC* in response to changes in diet or hormonal milieu. An exciting recent development is the demonstration that one of the conditional hypomorphic neural

enhancer alleles can be converted to full function in the adult CNS by tamoxifen administration to mice that are homozygous for the FloxNeo Δ nPE2 allele and intercrossed to a CAAG-CreERT (Cre recombinase estrogen receptor binding domain fusion protein) strain. They are now studying serial changes in body composition that occur in these mice over a short time period to determine if their weight loss is associated with differential changes in fat and lean body mass, decreased food intake, and/or increased basal metabolic rate and locomotor activity. In a third project, Dr. Low's lab is continuing to investigate the role of hypothalamic SST in mediating the sexually dimorphic phenotypes characteristic of the hypothalamic-pituitary growth hormone (GH) and hypothalamic-pituitary (ACTH)-adrenal neuroendocrine axes. A final common pathway for male/female differences in SST's ultimate physiological effects appears to be the sexually dimorphic patterns of hepatic gene expression. Additionally, experiments conducted with a strain of SST-deficient mice confirmed an important paracrine role for somatostatin in the regulation of both insulin and glucagon secretion from isolated pancreatic islets in response to metabolic and hormonal signals.

In collaboration with Dr. Martin Myers, these investigators generate novel reporter strains of transgenic mice for the analysis of neuronal polarity (axons vs. dendrites) in specific peptidergic subpopulations of hypothalamic neurons. They plan to study the functional distinctions between the subpopulations of POMC neurons that do or do not express leptin receptors. Dr. Low's research projects will be heavily supported by the Transgenic Animal Model Core, the Vector Core and the In Vivo Studies Core.

Another investigator interested in the physiology and pathophysiology of the hypothalamus as it relates to satiety and energy balance is Dr. Liang-you Rui. His laboratory investigates how the hypothalamus senses and integrates the various metabolic signals generated by hormones (e.g. leptin and insulin) and nutrients (e.g. glucose and lipids). He hypothesizes that these functions may be modulated by genetic and environmental factors; impairment in these sensory mechanisms may result in energy imbalance and obesity.

SH2B1 is a cytoplasmic adapter protein which binds via its Src homology 2 (SH2) domain to a variety of protein tyrosine kinases, including JAK2 and the insulin receptor. SH2B1-deficient mice are obese and diabetic. In a recent study, Dr. Rui showed that systemic deletion of the SH2B1 gene results in metabolic abnormalities in SH2B1 (KO) mice. These include hyperphagia, obesity, hyperglycemia, insulin and leptin resistance, and glucose intolerance. Neuron-specific restoration of SH2B1 beta not only corrects the metabolic abnormalities in SH2B1 (TgKO) mice, but also improves JAK2-mediated leptin signaling and leptin regulation of orexigenic neuropeptide expression in the hypothalamus. Moreover, neuron-specific overexpression of SH2B1 dose dependently protects against high fat induced leptin resistance and obesity. These observations suggest that neuronal SH2B1 is essential for controlling energy and glucose homeostasis (43). In the coming years, Dr. Rui plans to collaborate with Drs. Myers and Owyang to further investigate other molecular defects in the hypothalamic neural circuits causing energy imbalance and obesity. (Dr. Rui will utilize the Transgenic Animal Model Core and the Vector Core of the Center to facilitate his research.)

Transcriptional coactivators and corepressors are emerging as important regulators of energy metabolism and other biological processes. These factors exert their effects on the transcription of target genes through interactions with selective transcription factors and the recruitment of chromatin-remodeling complexes. Recent genetic and

biochemical analyses of the peroxisomal proliferators-activated receptor- γ coactivator 1 networks provide novel mechanistic insights regarding their role in the control of mitochondrial oxidative metabolism. These coactivators integrate tissue metabolic functions in response to nutritional signals as well as circadian timing cues. For example, Dr. Jiandie Lin, a Center investigator, in a recent article published in *Nature* (44) showed that PGC-1 α (*Ppargc1a*), a transcriptional coactivator that regulates energy metabolism, is rhythmically expressed in the liver and skeletal muscle of mice. PGC-1 α stimulates the expression of clock genes, notably *Bmal1* (*Arntl*) and *Rev-erba* (*Nr1d1*), through coactivation of the ROR family of orphan nuclear receptors. Mice lacking PGC-1 α show abnormal diurnal rhythms of activity, body temperature and metabolic rate. The disruption of physiological rhythms in these animals is correlated with aberrant expression of clock genes and those involved in energy metabolism. Analyses of PGC-1 α -deficient fibroblasts and mice with liver-specific knockdown of PGC-1 α indicate that it is required for cell-autonomous clock function in liver oxidative metabolism. These studies have uncovered a potential molecular target that could simultaneously moderate circadian clocks and energy metabolism. Disruption of the circadian rhythms has been implicated in the pathogenesis of metabolic disorders such as hepatic steatosis. (Dr. Li plans to utilize the Molecular Biology Core and the In Vivo Core for his research projects.)

In contrast to coactivators, transcriptional corepressors have been demonstrated to play an opposite role in the control of mitochondria biogenesis and respiration. The balance of these coactivator and corepressor proteins and, more importantly, their access to specific transcriptional partners are predicted to dictate the epigenetic states of target genes as well as the metabolic phenotype of the cells in health and disease conditions. In the coming years, Dr. Lin in collaboration with other Center investigators will continue to investigate the biological roles and mechanistic basis of the peroxisomal proliferators-activated receptor- γ coactivator 1 networks in the regulation of chromatin-remodeling and mitochondrial oxidative metabolism (45).

(b) Mechanism of visceral hypersensitivity

Visceral hypersensitivity is a key factor in the pathophysiology of gastrointestinal functional disorders such as non-cardiac chest pain, non-ulcer dyspepsia, and painful Irritable Bowel Syndrome. Despite the obvious clinical significance, there still remain much to be learned about the sites and mechanisms responsible for this abnormality. Center investigators including Lori Isom, John Wiley, Ying Li, and Chung Owyang will continue their research to enhance our understanding of the pathways involved in mediating visceral hypersensitivity at a cellular and clinical level.

Dr. Lori Isom's laboratory is well known for its work on the structure and function of voltage-gated sodium channels in health and disease. Sodium channels play a fundamental role in initiation and propagation of action potentials in excitable cells, including primary sensory neuron pain transmission. Recent contributions have focused on the role of sodium channel subunits as cell adhesion molecules (CAM). This body of work has focused primarily on beta subunits which interact both with the CAM contactin, as well as, the cytoskeletal protein ankyrin. Dr. Isom's published work suggests that these interactions are beta subunit-specific (33, 46).

Using a combination of gene targeting techniques with cell biological, electrophysiological, and molecular biological approaches, Dr. Isom further defined the

role of β subunits in neuronal excitability in vivo. She showed that in Scn2b (β 2)-deficient mice, there is a 40-50% reduction in the level of cell surface Navs in the central and peripheral neurons. The loss of β 2 results in significant decreases in Na^+ current density in hippocampal and dorsal root ganglion neurons. Most interestingly, Dr. Isom found that Scn2b null mice have reduced severity of pain symptoms suggesting that the absence of β 2 may be neuroprotective via preventing the axonal upregulation of Nav1s normally observed following demyelination. Dr. Isom hypothesizes that loss of β 2 subunits results in reductions in the level of cell surface Nav1s under basal conditions and attenuates the up-regulation of cell surface Nav1s in response to demyelination. This, in turn, prevents or reduces axonal degeneration. Hence targeting subunits may be a future therapeutic approach to the prevention and treatment of diabetic sensory neuropathy and visceral hyperalgesia.

Dr. John Wiley's laboratory investigated how chronic psychological stress affects the functioning of dorsal root ganglia neurons innervating the colon resulting in visceral hyperalgesia. This is an important area of research since it is well recognized that chronic stress frequently exacerbates symptoms of functional bowel disorders. Dr. Wiley's laboratory recently demonstrated that reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in water avoidance (WA) stressed rats. They showed that WA stressed rats have a significant increase in serum corticoidsterone levels and hypersensitivity to colorectal distension supporting stimulation of the hypothalamic-pituitary-adrenal (HPA) axis. In these rats, the endogenous CB1 agonist anadamide was increased significantly in the dorsal root ganglia. This is accompanied by a marked decrease in CB1 and a reciprocal increase in TRPV1 expression and phosphorylation in the DRG neurons. These reciprocal changes in CB1 and TRPV1 are reproduced by treatment of control dorsal root ganglia with anadamide in vitro. On the other hand, treatment of control dorsal root ganglia in vitro with the CB1 receptor antagonist WIN55, 212-2 decreases the levels of TRPV1 and TRPV1 phosphorylation. Treatment of WA stressed rats in vivo with WIN55, 212-2 or the TRPV1 antagonist capsaizepine blocks the upregulation of TRPV1 and prevented the development of visceral hyperalgesia. These novel observations suggest that CB1 and TRPV1 pathways may play an important role in stress induced visceral hyperalgesia. These may be potential therapeutic targets for patients with functional bowel disorders.

In the coming years, Dr. Wiley's laboratory plans to employ a combination of selective pharmacologic and gene silencing (siRNA) methodologist to test the hypothesis that targeted interventions at the levels of CB1 and TRPV1 receptors will prevent the development of visceral hyperalgesia in WA stressed rats. These investigators will further confirm that this phenomenon is mediated by corticoidsterone-mediated stimulations of endocannabinoid resulting in downregulation of CB1 and upregulation of TRPV1 receptor expressions and functions. They are going to show that there are both direct and indirect interactions which will involve PKC/PKA-dependent phosphorylation, and calcineurin-dependent dephosphorylation. The central hypothesis is that under basal condition, CV1 and TRPV1 have a direct interaction via formation of hetero-dimer and tetramer receptor complexes between CB1 and TRPV1 receptors which inhibits TRPV1 functions. They hope to demonstrate that elevated endocannabinoid levels on CB1 and TRPV1 receptors causes dissociation of the hetero-dimer and tetramer receptor complexes between these receptors and consequently release the inhibitory effects of CB1 and TRPV1 functions. This hypothesis will be tested using immunoprecipitation, laser confocal microscopy and electrophysiological studies.

These investigators also plan to demonstrate the “indirect” effects of elevated endocannabinoids on CB1 and TRPV1 receptors are mediated by cAMP dependent kinase and PKC-dependent phosphorylation of TRPV1 receptor via calcineurin-associated pathways. These series of studies will be facilitated by the four major cores of the Center (Molecular Biology, Cell Biology and Imaging, Proteomics, and In Vivo Cores).

The anterior cingulate cortex (ACC) is functionally related to cognitive, emotional, and affective processing of sensory information. Drs. Li and Owyang recently demonstrated that visceral hypersensitivity is at least in part mediated by the glutamatergic pathways involving metabotropic glutamate receptors in the ACC neurons. Single ACC neuronal discharges were recorded in response to colorectal distension in control rats and in two models of visceral hypersensitivity. They further showed that injecting the glutamate receptor antagonist MCPG into the ACC nucleus completely blocks the ACC responses to colorectal distension in visceral hypersensitive rats (47). In a subsequent study, these investigators demonstrated the hyperexcitability of the ACC neurons in visceral hypersensitive rats is due to an upregulation of the NR2B subunit of the NMDA receptor in these neurons (48). This novel observation may contribute to our understanding of enhanced nociceptive perceptions in patients with Irritable Bowel Syndrome. Conceivably, future therapeutics in IBS may involve the use of metabotropic glutamate receptor antagonists.

In subsequent studies, Drs. Li and Owyang, used a visceral hypersensitive model of colonic anaphylaxis evoked by intraperitoneal injection of chicken egg albumin and showed that ACC sensitization persisted for at least 2 months even though colonic mucosa inflammation had subsided within the first week. The persistence of ACC sensitization is mediated by changes in the strength of synapse such as long-term potentiation (LTP) in the ACC neurocircuits. This mechanism appears to be important for learning and triggering pain memories in the visceral hypersensitive state.

In the coming years, Dr. Owyang plans to further dissect the neurocircuits and cellular mechanisms responsible for the persistence of ACC sensitization. ACC field potentials elicited by electrical stimulation of the medial thalamus will be used as a quantitative measure of synaptic strength. Theta burst stimulations will be used to induce long-term potentiation and it is expected that enhancement of the ACC neuronal firings following theta burst stimulation in control rats will be similar to the increased response of ACC to colorectal distension in the visceral hypersensitive rats. These rats will also show enhanced visceral motor response to mechanical distension of the colon. Lesioning of different parts of ACC will be performed in an attempt to erase this “memory” which may allow identification of specific ACC regions important for learning and triggering pain memories in the visceral hypersensitive state.

Dr. Owyang also plans to investigate the molecular mechanism responsible for the induction and maintenance of long-term potentiation in the ACC synapses. He plans to show that repetitive visceral pain experience increases NMDA receptor in the ACC which in turn enhances long-term potentiation. Using a variety of techniques including electroporation of siRNA into the ACC to block the expressions of CaMK2, Dr. Owyang and colleagues hope to demonstrate that trafficking of NR2B to the post synaptic density (PSD) where phosphorylation of the subunit occurs in the visceral hypersensitive state. This is mediated by Ca²⁺/CaMK2 dependent signaling pathways. These studies will

require the critical support of the four major cores of the Center (Molecular Biology, Cell Biology and Imaging, Proteomics, and In Vivo Cores).

Clinical human studies will be performed to investigate visceral hypersensitivity in patients with IBS. These are collaborative projects among Drs. Owyang, Wiley and Morrow. They hypothesize that abnormal 5HT function participates in post prandial symptom generation in a subgroup of IBS patients with meal induced abdominal pain. These studies will involve brain imaging studies (MRI), spinal and cerebral evoked potential recordings, and pain behavior measurements. They plan to demonstrate that IBS-diarrhea (IBS-D) patients with meal induced symptoms have increased 5HT release compared to IBS patients without meal evoked complaints. This is due to the increased production or reduced 5HT inactivation by the gut serotonin reuptake transporters. Plasma 5HT parameters and colonic EC cell numbers, 5HT content and tryptophan hydroxylase-1 and SERT immunoreactivity will be compared between these two groups of patients. It is anticipated that the post prandial symptoms will be at least partially corrected by the administration of 5HT3 antagonist in those IBS patients with postprandial symptoms. At the same time, spinal and cerebral evoked potential during rectal distension will be compared in IBS-D patients with and without postprandial symptoms after 5HT3 antagonist administration. This study hopes to identify a subgroup of IBS-D patients who may be more responsive to the use of 5HT3 antagonist.

2. Molecular and Cellular Mechanisms and Inflammation

Inflammation is one of the major research themes shared by many Center investigators. Many research activities are directed at understanding the molecular and cellular mechanisms of inflammation, especially as it relates to Inflammatory Bowel Disease and *H. pylori* gastritis. These are common GI disorders in our clinical practice. In this new application, we have added a new Microbiome Core to provide Center investigators access to state-of-the-art technology for analysis of host-microbiome interactions. The following are highlights of some of the major research achievements in intestinal inflammation accomplished by Center investigators.

Dr. Steven Kunkel has a long established interest on cytokine and chemokine biology. His research activities are mainly directed at understanding the cytokine networks that are operative in a variety of inflammatory reactions and host defenses. Specific projects are on design to evaluate the expression and regulation of both proximal mediators, such as Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF-alpha), and more distal mediators such as Interleukin-8 (IL-8) and monocytes chemoattractant protein-1 (MCP). Dr. Kunkel and his colleagues have identified that macrophage-derived IL-1 and TNF-alpha can serve as early inflammatory gene products, which set in motion the cascade of events, resulting in the expression of chemokines from a variety of non-immune cells. This includes fibroblast, epithelial cells, hepatocytes, and endothelial cells. The ability of non-immune structural cells to express chemotactic cytokines is likely to play a fundamental role in host defense and provide mechanism for the localization of many acute and chronic inflammatory responses. While IL-1 and TNF-alpha appear to cause a proinflammatory response by initiating cytokine cascades, other cytokines may result in negative effects causing downregulation of cytokines and their cascades. These latter regulatory peptide mediators include interleukin-1 receptor antagonist, IL-4, IL-10, IL-13 and soluble cytokine receptors. During the evolution of chronic inflammation, the signals are likely to mediate the switch between maintenance of the response and progression to fibrosis to resolution of the inflammatory reaction (49-51). Future studies

will focus on epigenetic changes which may regulate the initiation, maintenance and resolution of acute and chronic intestinal inflammation. Over the years, Dr. Kunkel has helped to mentor and advise a number of Center investigators including Drs. Zimmermann, Higgins and Kao.

One of the research focuses of the Gumucio lab is on the functions of the epithelial cells as they relate to innate immunity. The regulation of intestinal immune responses is likely to play an important role in the pathogenesis of inflammatory bowel disease. The gut epithelium is critically involved in the maintenance of intestinal immune homeostasis—acting as a physical barrier separating luminal bacterial and immune cells, and also expressing anti-microbial peptides. Dr. Gumucio and her colleagues recently studied the molecular mechanism that controls this function of gut epithelial cells. They showed that the transcription factor NF- κ B, a master regulator of proinflammatory responses, functions in gut epithelial cells to control epithelial integrity and their interactions between the mucosa immune system and gut microflora. Intestinal epithelial-cell-specific inhibition of NF- κ B through conditional ablation of NEMO (also called $\text{I}\kappa\text{B}\gamma$ kinase-gamma) spontaneously causes severe chronic intestinal inflammation in mice. NF- κ B deficiency leads to apoptosis of colonic epithelial cells, impaired expression of anti-microbial peptides and translocation of bacteria into the mucosa. Concurrently, this epithelial defect triggers a chronic inflammatory response in the colon, initially dominated by innate immune cells but later also involving T-lymphocytes. Deficiency of the gene and coding the adapter protein MyD88 prevents the development of intestinal inflammation, demonstrating that Toll-like receptor activation by intestinal bacteria is essential for intestinal inflammation. Furthermore, NEMO deficiency sensitizes epithelial cells to TNF- α -induced apoptosis, whereas TNF receptor-1 inactivation inhibits intestinal inflammation, demonstrating that TNF receptor-1 signaling is crucial for disease induction. This study therefore identifies NF- κ B signaling in the gut epithelium as a crucial regulator of epithelial integrity and intestinal immune homeostasis. These findings may have important implications for understanding the mechanisms contributing to the pathogenesis of human inflammatory bowel disease (52).

In a separate but related study, Dr. Gumucio and collaborators provided evidence that PPAR- γ expression in mucosa epithelial cells is crucial for its anti-inflammatory effects with respect to colitis (53). These investigators generated mice with targeted disruption of the PPAR- γ gene in intestinal epithelial cells using a Villin Cre transgene and Floxed PPAR- γ allele and induce colitis by DSS administration. The mice showed increased susceptibility to DSS induced colitis with increased mRNA expression of proinflammatory cytokine such as IL-6, IL-1 β , and TNF. Interestingly, the PPAR- γ ligand rosiglitazone decreases the severity of experimental colitis and suppresses cytokine production in both PPAR- γ wild-type mice and mice with epithelial loss of PPAR- γ in the mucosa. These findings suggest that expression of PPAR- γ in mucosa epithelial cells is indeed crucial for prevention of colitis and for maintaining homeostasis of the mucosa barrier. The study also indicates that there are PPAR- γ independent pathways by which rosiglitazone exerts its anti-inflammatory potential during colitis. This observation paves the foundation for designing a clinical trial to study the effectiveness of PPAR- γ antagonist in patients with IBD. (Dr. Gumucio is a key member of the Microbiology Core and is a close collaborator of Drs. Merchant and Samuelson in their Program project DK062041.)

The Nunez laboratory is interested in signaling pathways regulating innate immunity as it relates the pathogenesis of IBD and cancer. Specifically, Dr. Gabriel Nunez's research focuses on investigating the role of proteins which act on nod-like receptor (NLR) and Toll-like receptor (TLR) families in mediating host immune response against microbial pathogens. His research employs a number of techniques including analysis of genetically modified mutant mice, cell biology and biochemical studies to examine the molecular mechanisms responsible for the interactions between microbial/endogenous molecules and NLR/TLR. In addition to inflammation, these signaling pathways may also play a role in cancer development. One of his recent studies demonstrated that the cytosolic sensors NOD1 and NOD2 are critical for bacteria recognition and host defense after exposure to Toll-like receptor ligands. They showed that NOD1 and NOD2 mediated signaling and gene expressions are enhanced in TLR-tolerant macrophages. Heightened responses to NOD1 and NOD2 stimulation are observed both in vitro and in vivo in mice made insensitive to TLRs. They showed that bacteria clearance upon systemic bacterial infection is critically dependent on NOD1 and NOD2 when mice are previously stimulated with lipopolysaccharide or *E. coli*. These findings indicate that activation of NLR signaling pathways that rely on cytosolic recognition of invasive bacteria serve to protect the host when proinflammatory responses are compromised by TLR-induced tolerance (54). (Dr. Nunez received important technical support from the Microbiology Core, Molecular Biology Core, and Cell Biology and Imaging Core. In return, Dr. Nunez has trained a number of young Center investigators including Dr. Grace Chen, who recently had become a full member of the Center. Dr. Nunez also collaborated with Drs. Zimmermann and Higgins.)

The Kao laboratory investigates the role of dendritic cells in innate immunity and intestinal inflammation. In a recent publication (55), Dr. Kao and colleagues used a transgenic mouse model which transiently removes dendritic cells (CD11c-DTR/GFP mice) and showed that these mice fail to induce significant inflammation upon exposure to dextran-sulfate sodium chemical irritants known to cause acute colitis. In control mice dextran-sulfate sodium induces profound production of proinflammatory cytokines (IL-12 and TNF- α) and chemokines (KC, MIP-1 α , MIP-2 and MCP-1). Ablation of dendritic cells markedly attenuates these proinflammatory responses. On the other hand, adoptive transfer bone marrow-derived dendritic cells greatly accentuates the severity of colitis. Furthermore, they showed that dendritic cell ablation is more effective than biologics (cytokine neutralizing antibodies) which is the current mainstay treatment for IBD. Dr. Kao believes targeting dendritic cells may be more effective than targeting T cells or cytokines, liken the analogy of turning off the faucet instead of mopping up the floor. If confirmed in humans, dendritic cell targeting therapy may represent an effective approach in treating patients with IBD. In future studies, Dr. Kao plan to study the interaction between micro and dendritic cells in the regulation of mucosa immunity.

Another promising research discovery by the Kao lab is the immunomodulatory role of HP infection on the development of inflammatory bowel disease. *H. pylori* is a highly prevalent, worldwide infection as the bacteria colonizes the gastric mucosa of half of the world's population because the incidence of IBD in regions of high *H. pylori* prevalence, which correlates with the initiation of anti-*H. pylori* therapy. One potential explanation is that *H. pylori* colonization is protective against IBD. In a meta analysis published recently in *Inflammatory Bowel Disease* (56). Dr. Kao showed that there is a significant negative correlation between the incidence of *H. pylori* infection and IBD suggesting a possible protective role (relative risk of 0.64) of *H. pylori* against the development of IBD. To further investigate this interesting clinical observation, he has started to investigate the

mechanism by which *H. pylori* modulates IBD risk. They showed that *H. pylori* directs the toleragenic programming of dendritic cells in a TGF-beta dependent fashion. Furthermore, *H. pylori* DNA genome harbors immunoregulatory sequences which suppress acute colitis induced by dextran-sulfate sodium (57). This finding if confirmed may support the hypothesis that global vaccination for *H. pylori* may lead to the further rise in the incidence of IBD. (Dr. Kao's research has been greatly facilitated by the Molecular Biology Core, Cell Biology and Imaging Core as well as the In Vivo Core.)

Gut microbiota plays an important role in the maintenance of mucosal immune homeostasis in the intestine and one potential mechanism is through the production of short-chain fatty acids, butyrate being the most abundant bioactive in the gut. A study published in *Gastroenterology* by Dr. Juanita Merchant examined how butyrate participates in ZBP-89 recruitment of ATM^{Ser1981} to induce p21^{waf1} in the maintenance of mucosal homeostasis. Reduction of ZBP-89 or ATM blocks butyrate-induced p21^{waf1} expression. In fact, mice with mutated ZBP-89 are found not to phosphorylate ATM^{Ser1981} in the mucosa and these mice were previously shown to develop more severe acute colitis after dextran-sulfate sodium treatment. These findings indicate a critical role of butyrate in mucosal homeostasis and may provide a symbiotic rationale for the existence of microbiota in host gut environment (58).

Other Center investigators who are involved in intestinal inflammation research include Drs. Yatrik Shah, Peter Higgins, and Ellen Zimmermann. Dr. Shah was recruited in 2008 as an Assistant Professor to the Department of Physiology, University of Michigan. He is an accomplished investigator interested in the molecular mechanism by which oxygen-sensing transcription factors regulate gastrointestinal mucosa homeostasis and inflammation. His recent study showed the hypoxia-inducible factor (HIF) is a critical regulator of barrier protection during colon epithelial injury. With the use of cre-lox-P technology, intestinal-specific disruptions of Von-Hippel-Lindau tumor suppressor protein (Vhl), HIF-1 alpha and laryl-hydrocarbonnuclear translocator (Arnt) is generated. He showed that colonic epithelial disruption of Vhl results in constitutive expression of HIF, which causes an increase in inflammatory infiltrate and edema in the colon. This effect is ameliorated in mice with disruption of both Vhl and Arnt/HIF 1 beta (which inactivates HIF). Disruption of both Vhl and Arnt in the colon epithelium also reduces clinical symptoms and histological damage in DSS induced colitis model. In addition, constitutive activation of HIF shows increased expression of proinflammatory mediators which is reduced by HIF targeted gene deletion. This observation showing that a chronic increase in HIF signaling in the colon epithelial cells initiates a hyperinflammatory reaction (59) may have important implication in developing therapeutic strategies for IBD. Dr. Shah currently is a recipient of a pilot feasibility study from the Peptide Center.

Dr. Peter Higgins, a NIH-supported junior investigator, has also received pilot feasibility study support from the Peptide Center. His research is focused on the molecular mechanism responsible for intestinal fibrogenesis in Crohn's disease. His recent study showed spironolactone and inhibitors of the rennin-angiotensin-aldosterone system (RAAS), are potent inhibitors of fibrosis in vitro. They appear to act by a mechanism independent of TGF beta transcription and independent of PXR and FXR nuclear receptors. In the coming years, Dr. Higgins plans to continue identifying the critical cells, or combination of cells, required for the induction of intestinal fibrosis. FACS flow cytometry and use of markers of fibroblast and myofibroblast phenotypes, including vimentin, actin, desmin and markers of immune activation, would help to characterize

the cellular changes in the intestinal wall. In this way, the subtype of cells responsible for synthesis of excessive extracellular matrix, and/or inhibition of degradation of the matrix will be identified. This approach will allow determination of whether cell types can act alone to produce fibrogenesis or whether it is a cooperative process between cell types, with paracrine signaling. These observations may be important for designing anti-fibrotic therapeutics in patients with Crohn's disease.

Dr. Ellen Zimmermann, another clinical investigator supported by NIH, is interested in developing new technique such as magnetization transfer MRI (mtMRI), which is able to detect tissue stiffness and potentially may be able to distinguish chronic intestinal inflammation from fibrosis. Using an intestinal fibrosis rat model (peptidoglycan-polysaccharide), Dr. Zimmermann shows mtMRI can quantitatively measure tissue collagens and is not sensitive to acute inflammatory changes. This preliminary data supports the use of this technology for monitoring the natural history of Crohn's disease. Its abilities to distinguish fibrotic from inflammatory strictures may have important diagnostic and therapeutic implications in Crohn's disease. Meanwhile, Dr. Zimmermann continues to collaborate with Dr. Gabriel Nunez in a clinical study to determine if NOD2 mutations are associated with strictures or other findings identified by CT enterography, as detailed in a previous section (Human studies).

Recent research indicates that the gut microbiota plays an important role in the regulation of energy metabolism, epithelial proliferation, innate immunity and visceral hypersensitivity. There is strong evidence that the gut microbial community represents a stable ecosystem which follows community "assembly" rules that govern the establishment and stability of these microbial consortia. Abnormalities in the microbiota may lead to a number of clinical disorders such as obesity, colonic neoplasm, chronic intestinal inflammation (IBD) and irritable bowel syndrome (IBS). In this application, we plan to add a new Microbiome and Inflammation Core to provide Center investigators state-of-the-art technologies to investigate the interactions within the endogenous microbiota and between the microbiota and the host in regulating mucosal responses in health and disease. The proposed core will provide state-of-the-art services to Center members. These include i) nucleic acid isolation; ii) terminal restriction fragment length polymorphism (T-RFLP) analysis; iii) 16S clone libraries; iv) 454 pyrosequencing; v) germ-free gnotobiotic mouse facilities and vi) expression of inflammatory/regulatory cytokine and genes. Drs. Gary Huffnagle and Vincent Young will be Co-Directors to lead the Microbiome Core.

Dr. Huffnagle's research focuses on host-microbe interactions as it relates to innate immunity. His laboratory has two major research programs: One project is to demonstrate that gastrointestinal inflammation (acute or chronic) produces immunoregulatory changes that affect the development and/or manifestation of immune response at distal mucosal sites including changes in regulating T cell responses and myelopoiesic. The second project is to determine the role of *Candida* species in modifying the resistance and resilience of the bacterial community structure in the gastrointestinal tract in response to ecologic stress or perturbation. Previous studies from other investigators have used selective plating techniques for analyzing the bacterial populations. Unfortunately, these approaches will underestimate or miss changes in the bacterial microbiota because 60-80% of bacterial species and greater than 99% of the total number of bacteria cannot be cultured. Dr. Huffnagle and his team will address this problem by using high-throughput pyrosequencing technology and other non-biased culture-independent techniques to analyze bacterial community structure.

Their preliminary data clearly demonstrate that the presence of *Candida albicans* makes a dramatic difference in the recovery of the bacterial community after disturbance. Thus, *Candida albicans* may exert a "keystone species effect" on the bacterial communities within the gastrointestinal tract.

Dr. Vincent Young, who has a national reputation in applying the technique of metagenomic analysis to study infection in the GI tract will co-direct the Microbiome Core. He has embarked on a series of experiments examining the role of the gut microbiota in the pathogenesis of *Clostridium difficile* infection. He is using a recently described murine model of *C. difficile* infection to conduct these experiments and is establishing a collaboration with other investigators to take these observations and use them to guide parallel studies in human patients with *C. difficile* infection.

Dr. Young has several other research projects that are leveraging his ability to conduct detailed examination of gut microbial ecology. His R01 grant "Microbial ecology of *Helicobacter*-induced colitis" (DK070875) examines the role of the gut microbiota in modulating colitis in IL-10^{-/-} mice that is triggered by infection with *H. hepaticus*. Part of this project involves detailing the changes in the community structure of the gut microbiota induced by antibiotic treatment. Dr. Young is also the PI for a multi-investigator grant that is part of the Human Microbiome Project (HMP), "The role of the gut microbiota in ulcerative colitis" (DK083993). This project involves following the establishment of the microbiota in patients who have undergone ileal pouch anal anastomosis for treatment of ulcerative colitis. An attempt is being made to determine if particular gut communities are associated with the development of pouchitis in these patients. Dr. Young is a PI on another multi PI grant that is funded as part of the HMP entitled "Cultivation and characterization of microaerobes from the human microbiome" (HG004906). This study uses state-of-the-art cultivation techniques combined with molecular profiling to identify and isolate novel bacteria from the gut microbiota that grow in the micro-oxic zone near the gut mucosal surface.

3. Cell Growth, Differentiation and Programmed Cell Death

(a) Cell growth and differentiation

Recognizing that mechanisms of adult GI disease often reflects misregulation of pathways used during embryonic and fetal development, the Peptide Center has continued to provide strong support to a group of investigators including Drs. Juanita Merchant, Linda Samuelson, Deborah Gumucio, Andrzej Dlugosz, who are interested in studying the mechanisms underlying the cellular decisions of identity in the developing and adult stomach and intestine. With the Center support, Dr. Merchant initiated a Program project (DK062041, PI: J. Merchant) in 2002 which has been funded in the last 7 years. The central goals and the common themes of the renewed Program project is to understand how the gastric epithelium establishes and maintains its identity in the healthy state and how it responds at the cellular level during injury or disease. Three common investigative themes pervade all projects: i) involvement of hedgehog signaling in the maintenance of cellular identity and response to injury; ii) the role of inflammation as trigger for cellular metaplasia or proliferation; and iii) the initiating mechanisms underlying gastric tumor regeneration. New mouse models have been developed that collectively provide valuable shared resources to examine how perturbation of highly conserved developmental signaling pathways influence whether a gastric cell maintains its identity or shifts toward a metaplastic or tumor regenic phenotype (60). Dr. Gumucio

studied the possible role of hedgehog ligands in mediating the interferon gamma-stimulated proliferation of gastric progenital cells (61). Dr. Dlugosz will investigate mouse models in which hedgehog signaling can be directly modulated in specific cell types. His preliminary data indicates that aberrant hedgehog signaling in cells at the squamo-columnar junction can result in the growth of the dysplastic lesion and frank tumors. Since this site is a common location for human gastric tumors, this has major clinical implications, especially in the US, where the incidence of this gastric tumor is increasing. Dr. Merchant has been studying the expression and processing of hedgehog by the parietal cells and uncovered evidence that interferon gamma, a major proinflammatory cytokine, dramatically modulates the hedgehog signal that flows from the parietal cells (62). She has also found that gastrin regulates parietal cell differentiation through its ability to stimulate hedgehog gene expression and processing. Finally, she is studying hypochlorhydria and antral hyperplasia produced by expressing a hedgehog inhibitor in parietal cells; this model shows that hedgehog is required for normal parietal cell function in vivo (22).

Drs. Linda Samuelson and Andrea Todisco are two other Center investigators interested in the study of gastric parietal cell physiology and pathophysiology. Dr. Samuelson recently showed that Huntingtin interacting protein 1 related (Hip1r) an F-actin- and clathrin-binding protein is expressed in gastric parietal cells. This molecule is required for tubulovesicle formation and parietal cell survival (21). Dr. Todisco showed that bone morphogenetic protein-4 (BMP-4), a peptide synthesized by mesenchymal cells in the stomach, plays an important role in the induction and maintenance of a differentiated parietal cell phenotype (20). The work of Drs. Samuelson and Todisco are highlighted in a previous section (physiology and pathophysiology of gastric parietal cells).

In a collaborative project between Drs. Gumucio and Samuelson, they reported that intestinal neurogenin 3 directs differentiation of a bipotential secretory progenitor to endocrine cell, rather than goblet cell fate. Using the mouse villin promoter to drive neurogenin 3 expression in the GI epithelium, they examined the effect of neurogenin 3 on cell fate. Their studies showed that transgenic founder embryos displays increased numbers of cells expressing the pan-endocrine marker chromagranin A. Expression of several hormones and pro-endocrine transcription factors is increased in the transgenics indicating that neurogenin 3 stimulates a program of terminal enteroendocrine cell development. At the same time, there is a corresponding decrease in numbers of goblet cells. These findings suggest that neurogenin 3 can redirect the differentiation of bipotential secretory progenitors to endocrine rather than goblet cell fate (63).

(b) Neural stem cell renewal, differentiation and aging

Dr. Sean Morrison, Director of Stem Cell Research at the University of Michigan, has been a member of the Peptide Center for the past 10 years. His laboratory has been studying the mechanisms that regulate stem cell renewal differentiation and aging. Stem cells are self renewing multipotent progenitors that give rise to all of the other cells in particular tissue. The Morrison lab has developed a technique to prospectively identify and isolate enteric neural crest stem cell by flow cytometry. These cells are self renewing, compose only a few percentage of fetal gut cells and can be identified in the proximal gut of a rat model of Hirschsprung's disease post natally. Dr. Morrison's laboratory demonstrated neuronal development after injection into the embryonic distal

gut of Hirschsprung's rat in organ culture. This provides the foundation to build a distal enteric nervous system in individuals with Hirschsprung's disease, with the intent to develop a mechanism to limit the need for surgical resection.

For the last several years, Dr. Morrison's has focused his research on mechanisms that promote stem cell maintenance throughout life. Stem cells may lie dormant, but can be activated at particular life cycle stages following injury (64). These cells are controlled within restricted tissue microenvironments known as "niches". Until recently, niches were a theoretical concept strongly supported by the observation that transplanted stem cells survive and grow only in particular tissue locations. Considerable progress has been made in elucidating how the microenvironment promotes stem cell maintenance. Abnormalities in these regulatory mechanisms are likely to contribute to aging and tumor regeneration (65).

In a recent study, Dr. Morrison's laboratory showed that physiological Notch signaling is critical for the maintenance of undifferentiated neural progenitors. In addition, this molecule is also key for gliogenesis (66). In another study, Dr. Morrison and colleagues showed that signaling lipids, phosphatidylinositol 3, 5-bisphosphate (PI [3,5] P2) is a key molecule for survival of neural cells. Using a mouse mutant model lacking Vac 14, a regulator of PI (3,5) P2 synthesis. Dr. Morrison showed that cell bodies of affected neurons are vacuolated and selective membrane trafficking pathways, especially entosome-TO-TGN retrograde trafficking, are defective suggesting that the housekeeping lipids PI (3,5) P2 is critical for the maintenance and survival of neural cells (67).

(c) Molecular mechanisms for synaptic development

Dr. Hisashi Umemori joined the University of Michigan as an Assistant Professor in the Department of Biological Chemistry in 2006. The research focus of Dr. Umemori's laboratory is to investigate the molecular mechanisms responsible for synaptic development in the enteric nervous system. In 2008, he received Pilot Feasibility support from the Center. In a recent study, he has purified presynaptic organizers from developing brains and identified fibroblast growth factor 22 (FGF 22) and its close relatives, FGF 7 and FGF 10 as molecules that can promote differentiation of presynaptic nerve terminals which is critical for synaptic formation. He further demonstrated that these FGF molecules are expressed in the GI tract and are involved in the synaptic formations in the ENS. In FGF 22 knockoff mice, there is a marked reduction in synaptic vesicles aggregation in the ENS. To visualize the enteric nervous system, he mated FGF 22 KO mice with YFP mice and examined the ENS network in the progeny compared to the wild type. The neural network of the ENS in the mutant mice is disorganized with irregular and winding axons suggesting that FGF 22 is a critical molecule in the maturation and maintenance of the ENS. Dr. Umemori received important technical supports from the Molecular Biology Core as well as the Cell Biology and Imaging Core.

In a separate study, Dr. Umemori demonstrated that the formation of neural synapses requires an exchange of organizing signals between the synaptic partners. Using synaptic vesicle aggregation in cultured neurons as a marker of presynaptic differentiation, he and his colleagues purified candidate presynaptic organizers from the mouse brain. A major bioactive species is the extracellular domain of the signal regulatory protein SIRP-alpha, a transmembrane immunoglobulin superfamily member

localized at neural synapses. They further demonstrated that the organizing activity of SIRP-alpha is mediated by CD 47. This molecule acts in concert with FGF 22 to induce vesicle clusters and promote neurite branching and pattern neural synaptic formation (68).

(d) Cancer Stem Cells

Recent studies indicate that stem cells have a critical role not only in the generation of complex multicellular organisms, but also in the development of tumors. Cells with the properties of stem cells are integral to the development and perpetuation of several forms of human cancers (69). Eradication of the stem cell-compartment of a tumor may be essential to achieve stable, long-lasting remission, and even a cure of cancer. Advances in our knowledge of the properties of stem cells have made specific targeting and eradication of cancer stem cells a realistic expectation.

Center investigator Dr. Diane Simeone has started to identify cancer stem cells in different tumors. She and her colleagues performed flow cytometric analysis of fresh tumor tissues using specifically selected surface (e.g. CD) markers for the identification of rare cancer stem cell-suspected cell populations and subsequently analyzed their tumor-regenic potential in animal models. Using these tools they showed that in 6/6 human colorectal cancer tested, their ability to engraft in vivo in immuno deficient mice is restricted to a minority subpopulation of epithelial cell adhesion molecules (EpCAM) high/CD 44+ epithelial cells. Tumors originated from these cells maintain differentiated phenotype and reproduce the full morphologic and phenotypic heterogeneity of their parental lesions. Analysis of the surface molecule repertoire of EpCAM high/CD 44+ cells leads to the identification of CD 166 as an additional differentially expressed marker, useful for cancer stem cell isolation in 3/3 colorectal cancer tested. These findings validate the stem cell working model in human colorectal cancer and provide a highly robust surface marker profile for colorectal cancer stem cell isolation (70).

Dr. Simeone is also among the first to identify pancreatic cancer stem cells using a xenograph model in which human pancreatic adenocarcinomas are grown in immuno compromised mice. She and her team identified a highly tumor-regenic subpopulation of pancreatic cancer cells, expressing the cell surface marker CD 44, CD 24 and epithelial specific antigen (ESA). Pancreatic cancer cells with this phenotype (0.2-0.8% of pancreatic cancer cells) have a 100-fold increased tumor-regenic potential compared with non-tumor-regenic cancer cells. This phenomenon is confirmed in an autotopic pancreatic tail injection model. The CD 24+ ESA+ pancreatic cancer cells demonstrate stem cell property of cell renewal, the ability to produce differential progeny, and increased expression of the developmental signaling molecule sonic hedgehog (71). Her laboratory plans to further investigate the signaling pathways that regulate the growth and survival of these pancreatic cancer stem cells. This type of research may provide novel therapeutic approaches to treat pancreatic cancer.

(e) Programmed cell death in health and disease

Another important research focus of the Peptide Center is on the mechanisms regulating programmed cell death. Research in Dr. Collin Duckett's laboratory focuses on the control of cell survival by the inhibitor of apoptosis (IAP) gene family, which encodes a group of factors with diverse cellular functions that range from suppression of apoptotic cell death by direct inhibition of apoptotic effector proteases to the control of mitotic spindle formation in conjunction with mitosis-regulated kinases. His research

focuses on x-link IAP (XIAP), a potent inhibitor of apoptosis that has been shown to play roles in apoptosis inhibition, cell cycle control, activations of stress-induced transcription factors and stress-activated kinases, common cytokine signaling, protein degradation through the ubiquitin pathway and even the metabolic control properties of XIAP. Through the characterization of XIAP-deficient mice, mutagenesis analysis, imaging studies of intracellular trafficking and the identification of novel XIAP-associated proteins, he is investigating the roles of XIAP in health and disease conditions (72-74). In a recent study, Dr. Duckett described an intriguing cytoprotective mechanism utilized by cellular IAP (c-IAP) and provides critical insight into how IAP proteins function to alter the apoptotic threshold in cells. c-IAP are highly unstable molecules that undergo auto-ubiquitination. This process is blocked upon co-expression with TNF receptor-associated factor (TRAF) 2 which inhibits the E3 ubiquitin ligase activity intrinsic to the RING of c-IAP 1. Loss of TRAF 2 results in decrease of c-IAP levels. Stabilized c-IAP is found to sequester and prevent SMAC/DIABLO from antagonizing XIAP and protects against cell death (75).

Working with Dr. Arjimand Mufti, a former GI research fellow, Dr. Duckett described a role for XIAP in copper metabolism. They found that XIAP levels are greatly reduced by intracellular copper accumulation in Wilson's Disease and in cells cultured under high copper concentration. Elevated copper levels cause a profound, reversible conformational change in XIAP due to the direct binding of copper to XIAP, which accelerates its degradation and significantly decreases its ability to inhibit caspase-3. This results in a lowering of apoptotic threshold, sensitizing the cell to apoptosis. Dr. Duckett's data provide an unsuspected link between copper homeostasis and the regulation of cell death through XIAP and this may contribute to the pathophysiology of Wilson's Disease (75).

Another key investigator involved in the research of cell survival and programmed cell death is Dr. Randall Kaufman. Dr. Kaufman's primary interest is to elucidate fundamental mechanisms and the physiological impact of intracellular signaling pathways that control protein synthesis and their fidelity of protein-folding in the endoplasmic reticulum (ER). In normal situations, upon accumulation of unfolded protein in the ER lumen, cells activate adaptive signaling pathways collectively known as the unfolded protein response (UPR). The UPR signals transient attenuation of protein synthesis to reduce protein-folding load and transcriptional induction of gene to expand the protein-folding and protein-degradative capacities of the ER. Abnormalities in this process appear to play an important role in a number of GI disorders such as hepatic steatosis and destruction of pancreatic beta cells resulting in diabetes (4, 76). For example, Dr. Kaufman and his colleagues showed that chronic ER stress promotes apoptosis, at least in part through the UPR-induced transcription factor C/EBP homologous protein (CHOP). In an elegant study recently reported in JCI (76), these investigators demonstrated that deletions of CHOP in multiple mouse models of Type II diabetes improve glycemic control and expand beta cell mass by promoting cell survival. In isolated islet from CHOP $-/-$ mice they found increased expression of UPR and oxidative stress response genes and reduced levels of oxidative damage. These findings suggest that CHOP is a fundamental factor that links protein misfolding in the ER to oxidative stress and apoptosis in pancreatic beta cells under conditions of increased insulin demand.

(f) In vivo molecular imaging for gene and protein expression (New Program)

To facilitate clinical application of the large number of recently discovered signaling molecules and biomarkers of neoplastic growth for the early detection of GI malignancy, the Peptide Center has launched a new in vivo molecular imaging program. Under the leadership of Dr. Thomas Wang and in collaboration with faculty from the School of Engineering, this program will provide the intellectual resources and infrastructure to design, develop and apply novel methods and systems for the early detection, diagnosis, treatment and monitoring of cancer and other GI diseases with targeted imaging, including wide area (mesoscopic) and confocal (microscopic) instruments. These instruments have the resolution spectroflexibility, and dynamic range to observer biology, and are particularly well suited to investigate the epithelium of the GI tract. Pre-clinical investigation of these methodologies includes cell-cell interaction, lymphocyte trafficking, and tumor migration. Screening of peptide candidates as surface markers will be performed using bacteriophage technologies. Clinical applications include detection of dysplasia in the esophagus and colon. The unique properties of light can be used to perform real time, high resolution imaging with levels of performance that far exceed other imaging modalities, such as MRI, CT, PET, SPECT, and ultrasound. Furthermore, optical methods can be used with molecular probes such as peptides to target specific biomarkers of disease to improve diagnostic sensitivity and specificity. This integrative approach can be used for early cancer detection, drug discovery, and monitoring of therapeutic response. The development of such imaging methods and contrast agents, including validation and translation, offers tremendous potential as a tool in the battle against cancer.

As an example, Dr. Wang and his team recently developed a probe for detecting colon cancer by screening phage display peptide libraries against fresh human colonic adenomas for high affinity ligand with preferential binding to premalignant tissue. They identified a specific heptapeptide sequence, VRPMLQ, which they synthesized, conjugated with fluorescein and tested in patients undergoing colonoscopy. They imaged topically administered peptides using a fluorescence confocal microendoscope delivered through the instrument channel of a standard colonoscope. In vivo images were acquired at 12 frames per second with 50 μm working distance and 2.5 μm (transverse) and 20 μm (axial) resolution. The fluorescein-conjugated bound peptide more strongly to dysplastic colonocyte than to adjacent normal cells with 81% sensitivity and 82% specificity. This methodology represents a promising diagnostic imaging approach for the early detection of colorectal cancer and potentially of other epithelium malignancies (77).

In collaboration with other Center investigators including Drs. Eric Fearon, Henriette Remmer, Joel Rubenstein, James Scheiman, and Chung Owyang, Dr. Wang has successfully competed for a Program project (U54 CA136429) directed at the in vivo detection of neoplasm in the GI tract. There are 4 specific aims which include i) to develop radio-labeled peptides to localize dysplasia on PET/SPECT/CT in the CPC/APC mouse model of colon cancer; ii) to demonstrate vertical cross-sectional imaging with submucosa tissue penetration depths using a miniature dual axis confocal microscope; iii) to select and validate peptide that affinity bind to high grade dysplasia in Barrett's esophagus; iv) to develop a miniature scanning fibroendoscope for wide area surveillance in the GI tract. Subsequently, in vivo studies in human to validate the clinical usefulness of the targeted peptides will be performed involving a network of academic centers including the University of Michigan, Mayo Clinic, University of Washington, and VA Palo Alto, California. The program will also have interactions with the industry including Olympus Medical System Corp, GE Healthcare, Mauna Kea Technologies, and STI Medical Systems Inc. Results

from initial clinical studies in patients with colon cancer and Barrett's esophagus will be used to plan a future multi-center clinical trial to begin at the end of the five-year funding period.