

## **ONGOING CENTER RESEARCH AND NEW INITIATIVE**

The Center is comprised of 55 primary Investigators who have demonstrated a mutual interest in its various activities and contributed substantially to its research base by participating in Core laboratories, serving on committees, or engaging in collaborative interactions with other Center Investigators. Although the Investigators of the Center have diverse research goals, a common thread of interest in research ties their laboratories on the biochemistry or physiology of the neurohormonal mediators of communication between different cells or organs of the body. Some Investigators focus on basic molecular research while others work 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 previous funding period the Center has brought its Investigators together in a network of collaboration that has broadened the horizons of each of the individual laboratories and moreover expanded interest in digestive disease related research. The specific research interests of the Center Investigators can be grouped into the following aggregates of projects with similar goals and objectives:

### **1. Transgenics and Gene Expression Profiling**

The Transgenic Core was added to the Peptide Center in the last renewal and many investigators have taken advantage of the effective service and discounted pricing to develop new animal models. Transgenic technology has become central to a broad array of research areas and over 40% of current Center Members have used transgenic or knockout models to examine physiological and pathophysiological roles of peptides and other regulatory molecules in health and disease. In addition, approximately one third of the Center Members have used the Transgenic Core to generate new rodent models. For example, the gastrin-knockout mouse model developed by the Samuelson laboratory has been an important resource for several investigators to study the significance of gastrin in whole animals. In addition to using this model to study mechanisms of regulation of gastric acid secretion, recent work in the Merchant laboratory has shown the development of gastric adenocarcinoma in gastrin-deficient mice, thus defining this mutant as an important gastric cancer model. Dr. Gumucio has described a villin promoter fragment that directs robust gene expression into the intestine, and with this tool has investigated the importance of morphogens like the hedgehog family for growth and development of the intestine. Dr. Thompson is one of the first investigators to take advantage of the transgenic rat production service in the Transgenic Core. He has developed a rat model expressing GFP in specific neurons in the brain. He is using laser capture microscopy to isolate these neurons and performing gene expression microarray analysis to obtain a molecular profile for the neurons under differing physiologic conditions.

Projections for the future have genetically engineered animal models playing an ever-increasing role in Peptide Center investigator's research.

Adenoviral vectors have also been an important technology for gene transfer into primary cells isolated from the gastrointestinal tract and other cultured cell models. Eleven different Peptide Center investigators have taken advantage of the Viral Vector Core for assistance with this technology. One project of note is the recent work published by Dr. Todisco using adenoviral-based gene transfer to define signaling pathways regulating growth factor action in isolated canine parietal cells. The Akt signaling pathway is central to controlling the growth and differentiation of parietal cells in culture. Adenovirus continues to be an important tool for genetic manipulation of gastrointestinal cells in culture and will remain a service in the Molecular Biology Core of this renewal proposal.

Gene expression profiling is another service that has been added to the Peptide Center over the past funding period because of the increasing demand for this technology among Center Investigators. The service core is effective and assists investigators in all aspects of the technology, including preparation of probes, hybridization and biostatistics analysis. This powerful technology allows thousands of gene transcripts to be examined, which allows an unbiased view of the cell physiology. Dr. Logsdon has used this technology to identify molecular signatures of pancreatic cancer and pancreatitis. These studies have led to the identification of possible new biomarkers for these diseases. Dr. Williams has used microarrays to profile changes in pancreatic gene expression under different physiological conditions to better understand the process of adaptation to diet. Dr. Gumucio is profiling transcription during intestinal development to understand the key signaling pathways regulating this process. Approximately 25% of Peptide Center Members have taken advantage of the Microarray Service in the past. Usage is projected to grow over the next funding period as investigators take advantage of this powerful technology.

## **2. Signal Transduction**

Center members carrying out studies of signal transduction continue to probe soluble intracellular messengers such as  $\text{Ca}^{2+}$  and cyclic nucleotides. However, most of the recent 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, glycogen synthase kinase (GSK) -3 and p70 S6K. Many pathways involve both protein phosphorylation and protein-protein interaction. A number of Center investigators are studying these pathways.

Several Center investigators have been at the forefront of identifying components of these pathways and protein-protein interactions in yeast or

cultured cells. This group includes K-L Guan (mTOR pathway), A. Vojtek (Ras, Akt), E. Stuenkel (SNARE Proteins) and prior to his departure J. Dixon (Tyrosine phosphatases, lipid phosphatases). A much larger group is studying the role of these pathways in differentiated cells of the GI tract. These studies are primarily focused on the pancreatic acinar cell (J. Williams, C. Logsdon, D. Simeone, C. Owyang), Gastric mucosa (A. Todisco, L. Samuelson, J. Del Valle, J. Merchant), intestinal mucosa (K. Bitar, D. Gumucio, E. Zimmerman) and neuronal cells of the autonomic nervous system (C. Owyang, M. Mulholland, S. Morrison, J. Wylie) and hepatocytes (L. Rui, O. MacDougald).

Examples of some of this work include J. Williams' demonstration of the importance of calcineurin in pancreatic growth, A. Todisco's studies of pathways regulating gastric mucosa differentiation and apoptosis, D. Simeone's studies showing that SMAD proteins bind to and regulate PKA and C.D. Logsdon's studies of the regulation of NF-KappaB in pancreatitis. These have often focused on either growth and differentiation or inflammation as the biological endpoint.

### **3. Pancreatic Physiology**

Investigators in the GI Center have continued to investigate pancreatic physiology. The Principal Investigators in this area have included Drs. Williams, Owyang, Li, Simeone and prior to his recent relocation, Logsdon. Associate Center members working on the pancreas include M. DiMagno and B. Ji. Drs. Li and Owyang have focused on the integrative neural and hormonal control of the pancreas with recent work centered on vagal control. Drs. Williams and Owyang have investigated cellular mechanisms controlling acinar cell secretion and Williams has begun a proteomics approach to the zymogen granule with the collaboration of P. Andrews and S. Ernst. The Williams lab has also defined the translational control mechanisms whereby digestive enzyme synthesis is regulated and begun studies on pancreatic growth. Drs. Simeone and Logsdon have provided new insights into the SMAD signaling pathway in acinar cells. This group of investigators has also pursued studies of pancreatic pathophysiology including experimental pancreatitis and pancreatic cancer.

### **4. Pathogenesis of Acute and Chronic Pancreatitis**

A number of investigators at the University of Michigan have a long established interest in pancreatic biology and pancreatitis. For example Dr. Matt DiMagno, working in collaboration with Drs. Owyang and Williams, has clarified the controversial role of nitric oxide in the early phase of experimental pancreatitis. His study demonstrated that eNOS derived nitric oxide exerts a protective effect on acute pancreatitis, likely through the action of endothelial cells to produce greater pancreatic blood flow. In separate studies these investigators showed that cystic fibrosis mice have increased

sensitivity to developing acute pancreatitis because of a baseline activation of pancreatic pro-inflammatory response secondary to an anti-apoptotic phenotype. Clinically these findings may explain the susceptibility to recurrent acute pancreatitis and chronic pancreatitis in patients with classic and non-classic cystic fibrosis.

Dr. Craig Logsdon in collaboration with Drs. Simeone and Ernst employed a unique approach using adenoviral-mediated gene transfer of an active subunit to directly activate NFB in the pancreas to show that activation of NFB alone is sufficient for induction of a pancreatic and systemic inflammatory response independent of trypsinogen activation. This data presents a new exciting and potential target, namely the NFB/IB system, for therapeutic intervention.

At the cellular level, Dr. Ernst and Williams have shown that cytoskeleton alterations are a common feature of acute pancreatitis. The increase in stress proteins, especially HSP27 and HSP70, appear to have a protective role against damage to the cytoskeletal system in the pancreas by noxious agents. Details of these findings are provided in the inflammation section.

## **5. GI Motility Studies**

The GI Motility research group received strong support from the Center for in vivo motility studies, utilizing virtually all the Core services provided by the Center. Some of the achievements are highlighted as follows: Orphanin FQ (OFQ) is a neuropeptide found in the central and enteric nervous system. Recently Dr. Owyang's laboratory has shown that OFQ is a potent stimulus of colonic motility, raising the possibility that analogues of OFQ may be used to treat constipation secondary to colonic inertia. In fact, in a subsequent study the Owyang laboratory demonstrated that an enhanced purinergic pathway occurs in postoperative ileus and that this could be reversed by OFQ. Using a rat model of post operative ileus provided by the In Vivo Studies Core, cecum manipulations reduced colonic muscle contractions which were reversed by administration of a P<sub>2</sub> purinoceptor antagonist. This was accompanied by an upregulation of colonic P<sub>2y1</sub> and P<sub>2y2</sub> receptors' gene and protein expression following abdominal surgery. At the same time there was increased ATP production in the myenteric neurons (Cell Biology and Imaging Core). In vivo studies showed that administration of OFQ, which is a potent inhibitor of purinergic transmission, reversed delayed colonic transit evoked by abdominal surgery. This is the first demonstration that an enhanced purinergic pathway occurs in post operative ileus, which likely contributes to the delay in colonic motility in these patients. The fact that the neuropeptide OFQ can reverse the condition suggests that it may have therapeutic potential.

To further characterize the OFQ receptor, Drs. Curro and Song with the assistance of the Molecular Biology Core have isolated the gene encoding this receptor in the rat. Their studies showed that rat OFQ receptor gene exceeds

10kb in length and contains six exons which are interrupted by five introns. The gene was alternatively spliced to yield multiple mRNAs. Unique regions in the intron 1 and in the 5' flanking region of the OFQ receptor gene were identified and appear to contribute to the regulation of its expression in different tissues. This provides the basis for designing specific agonists and antagonists of OFQ which may be useful to treat constipation and diarrhea conditions respectively.

In collaboration with Dr. Henry Mosberg, a peptide chemist with established interest in the pharmacology of opioids, Dr. Tsunoda identified the key amino acids that delineate OFQ and dynorphin A pharmacological selectivities on colonic contractions and transit. Using an in vivo rat model equipped with strain gauzes sutured to the colon, it was demonstrated that infusion of OFQ induced giant migrating contractions and accelerated colonic transit, whereas dynorphin A evoked non-migrating contractions and delayed colonic transit. Studies using OFQ peptide fragments with various amino acid substitutions showed that OFQ molecules contain a domain between amino acids 10 and 15 that excludes it from activating the opiate receptor. On the other hand dynorphin also contains an OFQ receptor "excluding" regions between amino acids 7 and 10 together with RSP<sup>8</sup>. To delineate the molecular interactions between OFQ-R, Drs. Tsunoda and Song transfected Cos 7 cells with normal or chimeric OFQ-receptor genes and performed binding and intracellular signal action studies. These together with 3D modeling predict the key amino acids of the OFQ receptor which are responsible for binding and signal transduction respectively. This information is important for designing agonists/antagonists of OFQ to treat colonic motility disorders. As a result of these studies Dr. Owyang has obtained 2 patents related to the use of OFQ in the treatment of motility disorders.

Gastric neuropathy in diabetes often results in defective accommodation and gastroparesis. Drs. Zhou, Wiley and Owyang recently reported that acute hyperglycemia increased nitro oxide synthase catalytic activity and mNOS gene and protein expression in normal rats. In a subsequent study these investigators further demonstrated that chronic hyperglycemia induced functional and structural damage to mNOS containing gastric neurons. This damage was mediated by endogenous nitric oxide which results in selective apoptotic cell death through the activation of NMDA receptors. This finding has therapeutic implications as the use of selective mNOS inhibitor and/or NMDA receptor antagonists may reduce or prevent nitrenergic neuropathy in diabetes.

Orexin, a neuropeptide present in the hypothalamus, is known to modulate feeding behavior. Drs. Yazdani, Wang and Owyang recently reported that orexin and its receptors are found in abundance in the enteric nervous system extending from the stomach to the colon. Orexin induces muscle contractions in the ileum via an intrinsic cholinergic pathway. Feeding dramatically

increases orexin-induced contractions through an upregulation of the orexin receptors. Thus it appears that orexin not only modulates feeding behavior, it also regulates intestinal motility according to the feeding status of the animals.

In a subsequent study Drs. Moises and Owyang reported that central administration of orexin may also modulate GI motility via the vagal pathways. Microinjection of orexin into the rostral part of the dorsal motor nucleus of the vagus (DMNV) produced an excitatory response on intragastric pressure. In contrast, microinjection in the caudal part of the DMNV produced an inhibitory response. Both the excitatory and inhibitory responses evoked by orexin were blocked by microinjection into the DMNV of nifedipine, an L-type  $Ca^{2+}$  channel blocker. Immunocytochemistry studies indicate there is a high density of orexin-1 receptors within the border of the DMNV that colocalize with acetylcholine-containing neurons. Parallel whole-cell voltage clamp recordings from DMNV neurons with gastric projections in primary culture showed that orexin modulated voltage-dependent  $Ca^{2+}$  currents in 79% of these neurons, producing facilitation or inhibition in roughly equal proportions of cells. DMNV neurons expressed at least 4 types of high threshold  $Ca^{2+}$  channels including L-, N-, r/q- and R-type channels. However, the modulatory effects of orexin on  $I_{Ca}$  could be accounted for by regulation of L-type  $Ca^{2+}$  channels. These indicate that voltage-gated  $Ca^{2+}$  channels are an important target whereby orexin acts postsynaptically to affect the excitability of the DMNV neurons. It shows for the first time that orexin can modulate  $Ca^{2+}$  signals by inhibiting or facilitating L-type- $Ca^{2+}$  channel activity. In this manner, orexin may act on the DMNV neurons projecting to the stomach to stimulate or inhibit gastric motility. Pilot data from this study helped Dr. Moises to successfully compete for a RO1 grant from the NIH.

## **6. Gastric Peptides and Acid Secretion**

Research in Dr. Andrea Todisco's laboratory focuses on the molecular events that are responsible for the growth promoting actions of the gastrointestinal hormone gastrin. Gastrin is known to have a variety of biological actions, chief among these is stimulation of acid secretion and regulation of the gut mucosal growth and proliferation. Although only the former action has been established clearly as physiologically relevant, the latter has received increasing attention because of the potential importance of gastrin as a growth factor, not only under conditions of normal development but also in pathological states such as gastric carcinoid tumors and colon cancer. Studies carried out in Dr. Todisco's lab have demonstrated that gastrin is a potent inhibitor of cellular apoptosis and that this effect requires the induction of both MAPK and protein kinase B/Akt through the activation of the small GTP binding proteins Ras, Rac and Cdc-42.

Dr. Samuelson has created a gastrin-deficient (GasKO) mouse model and has studied the physiology of acid secretion and cell biology of the gastric mucosa. GasKO mice have a major impairment in acid secretion and changes in the two major cell types responsible for gastric acid, parietal and enterochromaffin-like (ECL) cells. Recent studies have explored cell signaling in the parietal cells of control and GasKO mice and changes in gene expression using microarray analysis on purified parietal cells and fundic mucosa.

Studies on gastric peptides in the Merchant laboratory focus on the mechanisms regulating the transcriptional control of the hormone gastrin. Her lab has recently found with the assistance of Center Core facilities that gastrin is a target of the innate immune system. The studies on gastrin focus on promoter studies in cell lines and transgenic mice. She has recently found that the transcription factor AP1 cooperates with Sp1 another factor that we have previously identified as important in regulation of the gastrin promoter. Her plans are to determine if cooperation between these factors occurs in response to inflammatory cytokines, e.g., interferon gamma. Moreover, she has found that induction of gastrin by IFN $\gamma$  is not direct but rather may proceed through protease-mediated activation of EGF receptor ligands. She has recently generated a BAC clone that includes the human gastrin locus and has inserted this BAC into mice. Germline transmission has been documented and assessment of successful expression of human gastrin in mice is in progress. This will permit a more detailed analysis of the human gastrin promoter in an endogenous G cell setting.

## **7. Vagal Physiology and Digestive Functions**

Recent studies by Drs. Li, Tsunoda, and Owyang found that the vagal afferent pathways represent the primary targets by which peptide hormones and luminal factors stimulate pancreatic enzyme secretion. Using rat pancreatic physiology models, in combination with in vivo electrophysiology recordings, these investigators provide new insights into the neurohormonal regulation of pancreatic enzyme secretion. CCK at physiological doses acts via vagal cholinergic pathways to stimulate pancreatic secretion. In contrast to its effect on satiety, which is mediated by low affinity vagal CCK receptors, CCK acts through high affinity CCK receptors to evoke pancreatic secretion, suggesting that different affinity states of vagal CCK receptors mediate different digestive functions (25). In vivo electrophysiological studies of the nodose ganglia by Drs. Li and Owyang show that vagal afferent pathways also transmit sensory information about the mechanical and physiological state of the digestive tract mediated by serotonin (35,36). A synergistic interaction between CCK and serotonin at the level of the nodose ganglia may explain robust postprandial pancreatic secretion despite a modest postprandial increase in plasma CCK (37). Further studies using single vagal fiber recordings and immunocytochemistry mapping demonstrated that different

receptors on the nodose ganglia utilize different neural transmitters to send gastrointestinal sensory information to the CNS. Vagal primary afferent neurons activated by luminal serotonin contain glutamate and substance P, whereas those activated by secretin mainly contain CGRP immunoreactivity.

To delineate the central neurocirculatory of the vago-vagal reflex stimulated by luminal serotonin, Dr. Li utilized rats equipped with a chronic fourth ventricular cannula. The pancreatic nerve of the vagus was isolated and the efferent nerve activity to the pancreas was recorded. Combined electrophysiological and immunohistochemistry studies demonstrated that NPY, substance P, and orexin stimulated, whereas somatostatin inhibited the dorsal motor nucleus of the vagus (DMNV) preganglionic neurons (38). The interplay of these neuropeptides at the DMNV appears to play an important role in mediating vagal efferent signaling to the exocrine and endocrine pancreas.

Under the direction of Drs. Browning and Travagli the In Vivo Studies Core developed a technique to identify neurons from the nodose ganglion which innervate different regions of the GI tract. The anterogradely transported fluorescent tracer in DiI was applied to different regions of the GI tract in rats. After 3-4 days, primary cultures were made from dissociated neurons obtained from the left and right nodose ganglia. Whole cell patch clamp recordings were made using DiI-filled (identified) neurons. Using this technique, Drs. Browning and Travagli were able to demonstrate that the electrophysiological and pharmacological properties of identified gastrointestinal nodose sensory neurons innervating the GI tract differ according to the areas from which they received input. Such differences may provide the basis for regional variations in sensing transduction from the GI tract.

Currently, little is known about the neuropathway mediating the glucoregulatory effects of various gut peptides. With the assistance of the In Vivo Studies Core, Drs. Zhou and Owyang developed a rat model with cannula inserted into the dorsal motor nucleus of the vagus (DMNV) for microinjection into different regions of the nucleus. Euglycemia insulin clamp technique was utilized to maintain a steady blood glucose level. They found that GLP-1 directly acted on the intermedial portion of the DMNV to evoke insulin release via a cholinergic efferent pathway. In this manner, GLP-1 release from the distal small intestine in response to luminal carbohydrate such as in malabsorption states may enhance insulin release to prevent hyperglycemia.

Dr. Alberto Travagli demonstrated that adrenoceptors in the brainstem circuits play an important role in controlling gastric receptive relaxation in response to esophageal distension (39). In conjunction with Dr. Richard Rogers, Dr. Travagli developed a rat model to study neuropathways in the brainstem mediating receptive relaxation reflex in the esophagus of rats. This study

demonstrated NTS contains a large population of catecholaminergic neurons. In response to the esophageal distension, 53% of the tyrosine hydroxylase containing neurons in the NTS were activated, as evidenced by the demonstration of the c-fos immunoreactivity. In vivo studies in anesthetized rats showed that the 50% of the receptive gastric relaxation induced by esophageal distension was blocked by intracisternal application of either prazosin (1 adrenoceptor antagonist) or yohimbine (2-adrenoceptor antagonist). Combination of prazosin and yohimbine eliminated 75% of the reflex. These data suggest that adrenergic neurons of the NTS play a prominent role in the modulation of the gastric relaxation evoked by esophageal distension.

## **8. Human Studies**

During the current period, a significant amount of information with important clinical information has been gained through human studies. The human motility research group received invaluable support from the In Vivo Studies Core for their clinical investigation involving the use of PET imaging, evoked cortical and spinal potential studies, electrogastrography and barostat studies. In collaboration with Dr. Minoshima and subsequently Dr. Frey, Dr. Owyang performed PET imaging studies to examine cortical processing of sensory information evoked by gut stimulation in patients with irritable bowel syndrome. Enhanced stimulation of the anterior cingulate cortical and precortical regions was observed, and the intensity was markedly accentuated with simultaneous duodenal infusion of lipid but not carbohydrate or protein. The activation of these regions was not affected by 5HT<sub>3</sub> antagonist. This suggests that aberrant CNS processing of sensory information resulting from mechanical and chemical stimulation of the gut occurs in patients with IBS, and that this is not mediated by 5HT<sub>3</sub> pathways. Additional studies are planned with IBS patients to compare the brain foci activated by gut stimulation during diarrhea and constipation phases. Dr. Hasler also plans to perform similar studies to investigate neural pathways in the CNS mediating the perception of nausea and bloating. Using PET imaging studies Dr. Ladabaum has identified the cortical and subcortical structures activated by mechanical stimulation of the antrum. This provides the basis to study patients with nonulcer dyspepsia.

Dr. Chey and Wiley performed evoked cortical and spinal potential studies to localize anatomic sites by which a peptide exerts its inhibitory effects on the perception of visceral pain. Agents such as somatostatin and peripheral acting opioids markedly reduced evoked spinal potential evoked by colorectal distension, indicating a peripheral site of action. In contrast central acting agents such as the tricyclic compounds (mirtazapine) did not affect evoked spinal potential studies. Similar studies are planned to investigate the effects of 5HT<sub>3</sub> receptor blockade on evoked spinal potential induced by colorectal distension in diarrhea prone IBS patients.

With better electrode design and improved software, electrogastrography (EGG) has become a reliable tool for investigating gastric dysrhythmias, which are quite common among patients with diabetic gastroparesis. Results from studies by Drs. Owyang and Hasler showed that ginger, a Chinese herbal remedy, and nitric oxide donors significantly reduced nausea and tachygastria induced by hyperglycemia. Recently Drs. Coleski and Hasler have validated a technique which places electrodes in different regions of the stomach via endoscopy to map myoelectric activity arising from different areas in the stomach. This novel method provides a useful tool to map the origins of ectopic gastric pacemaker in gastria dysrhythmic conditions.

Working in conjunction with the Department of Bioengineering, the In Vivo Studies Core has come up with another innovative apparatus for gas collection, which has been used in conjunction with a barostat to evaluate the patterns of gas transit in the small bowel. This breakthrough technology allows monitoring the movement of gas in the intestine in IBS patients with gas and bloating, and evaluation of therapeutic agents.

Since the last renewal of the Center grant, a blood and tissue bank has been added to the In Vivo Studies Core. This new facility provides a vital service to Center investigators interested in obtaining blood and tissue samples for in vitro studies. For example, Drs. Zimmermann and Nunez obtained blood and intestinal samples from patients with Crohn's disease and ulcerative colitis in order to study the mononuclear cells' response to a bacterial protein muranyl dipeptide and related the results to NOD2 genotypes. Drs. Logsdon and Simeone received samples of pancreatic adenocarcinoma and chronic pancreatitis to perform molecular profiling studies using microarray technology. Drs. Owyang and Wiley obtained surgical stomach tissue from patients with refractory diabetic gastroparesis and performed detailed immunochemistry and electrophysiological studies of the enteric nervous system and the interstitial cells of Cajal. In addition, blood samples from type I and type II diabetic patients with or without diabetic neuropathy allowed Dr. Wiley to purify an immunoglobulin protein which appears to activate apoptosis of neurons. For details please see the section in the In Vivo Studies Core.

## **9. New Initiatives**

In the current proposal we would like to highlight three thematic areas that reflect the common research interests of numerous investigators affiliated with the Center. These areas include; (1) Neurobiology in Appetite Control 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 involved in regulation of gastrointestinal function and disease. Investigators exploring

each of these themes utilize a broad range of core resources provided by the Center.

**a. Neurobiology in Appetite Control and Visceral Pain**

**i. Appetite and Its Regulation**

Obesity is a major health problem worldwide. It may lead to a myriad of serious health problems, including heart disease, hypertension and diabetes. Thus understanding the complete mechanism regulating eating behavior has important clinical significance. A large group of investigators affiliated with the University of Michigan, Gastrointestinal Peptide Research Center have joined forces to characterize the pro- and anti-satiety pathways, their chemical coding and regulation. In addition, considerable effort has focused on the role of mediators of the stress response as potential modulators of appetite.

Dr. Ira Gantz's laboratory's major research focus is on the role of the melanocortin system in appetite regulation. The melanocortin system consists of melanocortin peptides derived from the pro-opiomelanocortin gene, five melanocortin receptors, two endogenous antagonists and two ancillary proteins. In collaboration with Drs. Watson and Akil and Mosberg, Dr. Gantz's laboratory performed research on the basic biochemistry, physiology and pharmacology of the melanocortin system and we will highlight progress made in these areas.

The first project examines the molecular pharmacology of the melanocortin-4 receptor (MC4R). This is a key receptor for the satiety factor alpha melanocyte stimulating hormone and the orexigenic factor agouti-related protein. Using three dimensional computer modeling and site directed receptor mutagenesis this project examines the ligand-receptor binding determinants and the molecular basis for the melanocortin receptor subtype specificity of agouti-related protein. Significant progress in identifying transmembrane and extracellular loop residues that are involved (directly or indirectly) in ligand binding has been made. They have also found that the binding determinants of agouti-related protein and alpha-melanocyte stimulating hormone, while very similar, are not identical. Future studies will continue the iterative process of mutagenesis and modeling using newly identified highly selective MC4R ligands. The second project is to identify mRNA regulated by fasting in the arcuate nucleus of the hypothalamus. Using oligonucleotide microarrays Dr. Gantz identified 321 candidate mRNA regulated by 48 hours of fasting. One of the mRNA, which he has validated, is called anykyrin repeat and SOCS-box containing protein 4 (Asb-4). Asb-4 is a protein previously named for its structural motifs, but whose exact function is unknown. Neuroanatomical studies demonstrate that Asb-4 is differentially expressed by pro-opiomelanocortin expressing and neuropeptide

Y expressing neurons, two neuronal cell types in the arcuate nucleus recognized as central to energy homeostasis. Moreover, Asb-4 mRNA is down-regulated by fasting and down-regulated in the arcuate nucleus of the fa/fa Zucker rat. The brain distribution of Asb-4 is strikingly limited to hypothalamic nuclei and areas and one amygdaloid nucleus known to be involved in energy homeostasis. Present experiments are designed to identify the protein(s) that bind Asb-4 and will use RNA inhibition to identify the Asb-4 function. The third project is to use oligonucleotide microarrays to identify hypothalamic mRNAs that are regulated by activation of the MC4R. In order to identify these mRNA Dr. Gantz plans to administer a highly selective tetrahydroisoquinoline MC4R agonist intracerebroventricularly to normal mice and MC4R knock-out mice. Four hours after stimulation the paraventricular nucleus, a hypothalamic area rich in MC4R, will be harvested using laser microdissection. mRNA harvested in this fashion will be used for the microarray study.

Collaborative studies involving Drs. Stan Watson's and Huda Akil's laboratories have provided a wealth of information on the role of serotonergic, dopaminergic, and opioid pathways in affective disorders which have well-described effects on appetite. Recently Drs. Watson's and Akil's laboratories have been instrumental in characterizing the expression and regulation of the orexin/hypocretin receptors (OX1R and OX2R) in the brain. The orexin peptide family appears to have an important role in the hypothalamus as a regulator of feeding behavior. Using quantitative in situ hybridization, they showed that expression of OX1R and OX2R mRNA exhibited distinct distribution patterns. The hypothalamus and amygdala are two primary sites for integration of a variety of appetite regulating signals in the brain. After 20 hours of fasting, levels of OX1R mRNA were significantly increased in the ventromedial hypothalamic nuclei and the medial division of amygdala. An initial decrease (14h) and a subsequent increase (20 h) in OX1R mRNA levels after fasting were observed in the dorsomedial hypothalamic nucleus and lateral division of amygdala. On the other hand levels of OX2R mRNA were augmented in the arcuate nucleus, but remained unchanged in the dorsomedial hypothalamic nucleus (DMH), paraventricular hypothalamic nucleus and amygdala following fasting. The time dependent and region-specific regulating patterns of OX1R and OX2R provide insight into the functions of feeding behavior. It is quite conceivable that the interconnections among the orexin receptive sites form distinct orexin-signaling circuits which may elicit sequential responsiveness to different feeding states.

In separate studies Dr. Akil and Watson's investigated the mechanisms by which  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ MSH) regulates feeding. The anatomical overlap and functional similarities between  $\alpha$ MSH and corticotropin-releasing hormone (CRH) led to the hypothesis that CRH might act as a mediator of the central actions of the melanocortin systems. By double-labeling in situ hybridization a subpopulation of CRH neurons in the

paraventricular nucleus of the hypothalamus (PVN) were shown to contain the melanocortin-4 receptor (MC4R), concentrated in the ventromedial part of the paraventricular PVN. Intracerebroventricular injection of the melanocortin agonist MT11 to conscious and freely moving rats induced a rapid induction of CRH gene transcription in the PVN. This effect was accompanied by a rise in plasma corticosterone levels in a dose and time-dependent manner. MT11-induced increase in plasma corticosterone was attenuated by the selective MC4R antagonist HS104 and non-selective CRM receptor antagonist  $\alpha$ -helical-CRHQ-4 in a dose-dependent manner. Moreover, the abhorrent effect of MT11 was markedly reversed by pretreatment with  $\alpha$ -helical-CRHQ-4. These findings provide evidence CRH acts as a downstream mediator of melanocortin signaling and contributes to the mechanisms by which the central melanocortin system controls feeding and neuroendocrine responses.

Dr. Owyang in collaboration with Drs. Li, Travagli and Moises performed in depth investigation of leptin and CCK in the regulation of satiety. Their studies indicate that the paradigm for control of feeding behavior has changed significantly. The separation of function – CCK control of short-term satiety and leptin regulation of long-term food intake and body weight – has become less clear. Research has shown that leptin, in the presence of CCK, may also mediate control of short-term food intake. This interaction occurs at the vagus as it is abolished by vagotomy-CCK-A receptor and leptin receptors colocalize in caudal but not rostral regions of human and rat nodose ganglia. Low-affinity CCK-A receptors couple to extracellular  $Ca^{2+}$ -dependent Sre, RhoA and P13K pathways, and leptin receptors couple to the JAK-STAT pathway. Activation of vagal CCK receptors enhances the leptin signal transduction pathway by amplifying the signaling of STAT-3 molecules. This synergistic interaction between the two satiety signal molecules CCK and leptin, in the nodose ganglia may serve to control short-term satiety. It is conceivable that abnormal regulation of these receptors or their signal transduction pathways may cause eating disorders resulting in obesity.

Dr. Audrey Seasholtz's laboratory is examining the molecular biology, biochemistry and physiology of corticotrophin-releasing hormone (CRH) in the regulation of the mammalian stress response. Dr. Seasholtz has employed transgenic and "knock-out" strategies to create mouse models of CRH-binding protein over-expression and deficiency. Of interest, the CRH-binding protein deficient mice demonstrate increased anxiety, decreased weight gain and decreased food intake compared to wild-type controls suggesting that increased free levels of CRH and other CRH-like peptides are involved in the pro anorexic and anxiogenic effects. In subsequent studies, Dr. Seasholtz showed that the CRH-binding protein is made in the brain and pituitary and appear to be constitutively secreted from many cell types. To further investigate the biology of CRH binding protein, Dr. Seasholtz made a fusion protein of CRHBP with EGFP. This CRHBP-eGFP fusion protein is readily visible by confocal microscopy and can be used to study the localization of

CRHBP in a variety of cell types and its movement in cells after presentation with ligand.

## **ii. Neural Peptides and Regulation of Visceral Pain**

Despite the obvious clinical significance, advances in visceral pain research have been plagued historically by inadequate quantitative methodological approaches. However, applications of recent advances from a variety of disciplines including molecular biology, cell biology and behavior research, have led to significant advances in our understanding of the pathways that transmit visceral pain and the role of neuropeptides in the regulation of these pathways. Research in this arena represents a major focus for a number of investigators of the Peptide Center, including Lori Isom, John Wiley, Stan Watson, Huda Akil, Chung Owyang, Ying Li, and Henry Mosberg.

At the molecular level, Dr. Isom's laboratory is examining the subunits of sodium channels subserving the transduction of extracellular signals into the cytoplasm. Her laboratory showed that TTX-insensitive sodium channels appear to be preferentially expressed on afferent neurons that transmit nociception. As a result from studies in her laboratory and others, the events underlining nodal formation, including sodium channel clustering, are now beginning to be understood. A complex interplay between a number of extracellular and intracellular signaling events is required. Investigators in her laboratory have shown that auxiliary subunits of voltage-gated sodium channels, beta 1 and beta 2, contain extracellular cell adhesion molecule domains. These proteins do not form the ion conduction pore of the channel and yet have significant modulatory effects on channel gating, voltage-dependence of channel activation and inactivation, and the levels of channel protein expressed at the plasma membrane. This study was among the first to show that an extracellular matrix protein secreted by oligodendrocytes during the early phases of myelination, tenascin-R, functionally modulates sodium channel beta 1 and beta 2 subunits, resulting in migration of beta subunit expressing cells away from TN-R substrates. Dr. Isom has also shown that beta subunits interact homophilically through the extracellular Ig domains to produce cellular aggregation via homophilic cell adhesion, resulting in recruitment of the ankyrin cytoskeleton to sites of cell-cell contact. Current projects investigate the signal transduction events triggered by beta subunit-mediated cell adhesion. Dr. Isom's laboratory is also investigating the molecular targeting signals encoded in the beta subunit amino acid sequences and how the membrane targeting of alpha subunits is modulated by beta 1 and beta 2. Recently she has completed beta 1 and beta 2 subunit gene knockout experiments, showing that the beta subunits are critical for maintenance of channel density at the cell surface and formation of the axo-glial complex at nodes of Ranvier. In addition to mouse models, Dr. Isom is also developing projects using zebra fish to study sodium channel genetics.

Dr. John Wiley's laboratory continues to work on novel cellular mechanisms that contribute to abnormalities in nerve function in diabetes. His laboratory demonstrated previously that calcium entry is increased in diabetic neurons and associated with activation of program cell death. This appears to be mediated by a specific immunoglobulin. The channels via which calcium enters the neurons are regulated by the G proteins. Dr. Wiley's laboratory has shown that the function of the G proteins inhibiting calcium channels is impaired in diabetes, thereby allowing abnormal amounts of calcium to enter the cell. The intracellular pathway that causes altered inhibitory G protein functions involves activation of protein kinase C which ultimately increases phosphorylation of the G protein, resulting in decreased function. This is the first demonstration in which an acquired disease alters G protein function. Better understanding of the process may lead to prevention and treatment of diabetic neuropathy.

In collaboration with Dr. Lori Isom, Dr. Wiley's laboratory demonstrated that early painful diabetic neuropathy is associated with differential changes in TTX-sensitive and TTX-resistant sodium channels in dorsal root ganglia neurons in streptozotocin induced diabetic rats. Further studies from Dr. Wiley's laboratory demonstrated that the functions of TTX-sensitive and TTX-resistant sodium channels are increased in early diabetic neuropathy. These enhanced sodium currents are correlated with increased phosphorylation at both serine/threonine and tyrosine sites. Using a new generation of specific inhibitors or blockers for TTX-sensitive and TTX-resistant sodium channels, Dr. Wiley hopes to elucidate the different subtypes of sodium channels in the development of hyperalgesia in painful diabetic neuropathy, setting the foundation for improved targeted therapeutic intervention.

In a related study Dr. Wiley's laboratory also reported that early painful diabetic neuropathy is associated with differential changes in the expression and function of vanilloid receptor 1 (VR1). In his recent studies, DRG neurons from diabetic rats showed significant increases in capsaicin and proton-activated inward currents. Capsaicin-induced desensitization of VR1 was downregulated, whereas VR1 re-sensitization was upregulated in this group of neurons. The PKC activator PMA blunted the VR1 desensitization, and this event was reversible in the presence of PKC inhibitor. Of interest, the tetrameric form of VR1 increased significantly in DRGs from diabetic rats. This was accompanied by increased phosphorylation levels of VR1. Co-localization studies demonstrated that VR1 expression was increased in large myelinated A-fiber DRG neurons whereas it was decreased in small unmyelinated C-fiber neurons as a result of diabetes. Hence this study demonstrated for the first time that painful diabetic neuropathy is associated with enhanced function of VR1 in DRG neurons. The enhanced function involves increased phosphorylation, oligomerizations, and reallocation of channels to cell surface plasma membrane. Impaired desensitization of VR1

may also contribute to regulation of the excitability of sensory neurons that mediate neuropathic pain. These findings provide a rationale for targeted therapeutic intervention to modulate VR1 expression and neural function.

It is now commonly believed that visceral hypersensitivity plays an important role in the pathogenesis of painful irritable bowel syndrome. The anterior cingulate cortex (ACC) is functionally related to cognitive, emotional, and affective processing of sensory information. Imaging of the human brain indicates that abnormal processing of visceral sensory signals by the ACC may aggravate irritable bowel syndrome. Drs. Li and Owyang recently examined the hypothesis that visceral hypersensitivity may sensitize ACC visceral stimulation, leading to a worsening of symptoms and functional bowel disorders. To test this hypothesis they recorded single ACC neuronal discharges in response to colorectal distension in control rats and in two new models of visceral hypersensitivity, induced by either intraperitoneal injection of chickenate albumin to evoke intestinal and colonic anaphylaxis, or intracolonic instillations of 0.6% acetic acid followed by three days of colorectal distension. None of the 78 ACC neurons recorded in controls responded to graded colorectal distension (10, 20, and 50 mm Hg). In contrast, up to 49% of the 41 ACC neurons tested in the visceral hypersensitive rat model responded with a robust firing in response to 20 mm Hg distension. Microperfusion by reverse microdialysis of the metabotropic glutamate receptor antagonist MCPG into the dendritic field and somata of the ACC completely blocked ACC responses to colorectal distension in the visceral hypersensitive rats. On the other hand, glutamate perfusion markedly increased ACC neural firing. Hence it appears that there is enhanced excitability of the anterior cingulate cortex in response to colonic distension in visceral hypersensitive rats. This is mediated by glutamatergic pathways involving metabotropic glutamate receptors in the ACC neurons. This mechanism may contribute to enhanced nociceptive perceptions in patients with irritable bowel syndrome. Conceivably, future therapeutics in painful IBS may involve the use of metabotropic glutamate receptor antagonists.

#### **b. Molecular and Cellular Mechanisms of Inflammation**

There are several laboratories that have Center-supported research related to inflammation. Research in Dr. Steven Kunkel's lab has centered on the role of cytokines as inflammatory mediators. He has maintained collaborative interactions with investigators studying *Helicobacter pylori* gastritis and the etiology of inflammatory bowel disorders. Related to the latter, Dr. Kunkel has served as a valued consultant for Dr. Ellen Zimmerman in her studies on cytokines in the pathogenesis of IBD. Additional laboratories using Center Cores have now made major advances in inflammation based research.

Perhaps the clinically most significant advance has been the cloning of the Nod2 protein by Center member Gabriel Nuñez. During the course of

studying factors involved in apoptosis, Dr. Nuñez discovered that one of these molecules was the long sought after targeted loci that is mutated and increases susceptibility to Crohn's disease. Two NOD proteins, Nod1 and Nod2, activate NF-kappaB and appear to be involved in the cellular response to bacterial pathogens. Ongoing studies of Nod1 and Nod2 proteins include molecular studies to further define their mechanism of action and analyses of mutant mice deficient in Nod1 and Nod2. Also the Nuñez laboratory has identified Bcl10/CIPER, a protein involved in the development of a subset of MALT B-cell lymphomas. MALT lymphomas are the subset of gastric cancers that arise in response to chronic *Helicobacter pylori* infection. Recent studies indicate that Bcl10 functions in a novel NF-kappaB signaling pathway induced by antigen receptor stimulation. Studies to characterize the Bcl10 signaling pathway are in progress.

Studies by Dr. Deb Gumucio have focused on understanding the molecular etiology of familial Mediterranean fever (MEFV). The protein product of the MEFV locus is pyrin, a protein expressed primarily in neutrophils and macrophages. Dr. Gumucio was part of the consortium that positionally cloned pyrin from blood samples from patients with MEFV. A yeast-two hybrid screen to identify pyrin-interacting proteins has identified two proteins that link pyrin to the cytoskeletal signaling and apoptosis pathways. These investigators are further exploring those links to elucidate the function of wild type pyrin in the cell and to understand how clinically-relevant mutations in pyrin affect those functions. The latter studies have been carried out in collaboration with Dr. Nuñez.

Studies in Dr. Teitelbaum's lab have focused on the working hypothesis that the administration of TPN results in significant changes in the phenotype and function of the intestinal intraepithelial lymphocyte (IEL) population - the population of lymphocytes lining the intestinal lumen - including an increased expression of interferon gamma. These changes in the IEL appear to play a key role in both the breakdown in mucosal barrier function, as well as the development of villus atrophy.

Dr. John Kao seeks to understand the role of dendritic cells in bacterial-host interaction and to understand the pathogenesis of chronic mucosal inflammation. Using *Helicobacter pylori*-induced chronic gastritis as a model of bacteria-induced chronic inflammation in the stomach, much of his current efforts are devoted to probing the mechanism of dysregulated adaptive host immune response to *H. pylori*. These studies have been carried out in collaboration with Drs. Merchant and Eaton. Understanding how bacterial pathogens escape host immune defense mechanisms is pivotal to elucidating the mechanisms of chronic persistent infection, which may be applied to other chronic viral infections such as hepatitis B and C viruses. Another area of interest with direct clinical implications is the use of dendritic cells as an adjuvant to stimulate protective immunity against various pathogens. Using a

mouse model of colon cancer in collaboration with Dr. J-J. Chen, Dr. Kao has demonstrated the effectiveness of dendritic cell vaccines in preventing colon cancer growth. He is interested in further investigating the mechanism of therapeutic failure in tumor-bearing hosts. It is hoped that this work will further uncover tumor-induced immune dysregulation that may enable the escape of tumor from immune surveillance.

The Eaton laboratory has been studying the pathogenesis of *Helicobacter pylori* since 1987 and was the first laboratory to use an animal model to define bacterial colonization factors. Her studies currently involve use of mouse models to study the role of host immune factors in the outcome of infection and the interactions between bacterial products and host immunity. Current areas of interest include the roles of CD4+ regulatory cells and dendritic cells in the induction of disease, the role of interferon-gamma and interleukin-10 in disease and protection, and the mechanism of disease induction by *H. pylori* lipopolysaccharide O-antigen.

Dr. Juanita Merchant's laboratory is studying the affect of inflammatory signals on the gastric mucosa. She has found that chronic inflammation occurring in gastrin-deficient mice (developed by Linda Samuelson) predisposes these mice to distal gastric cancer. In pursuing the molecular changes that result in neoplastic transformation, her lab has focused on two-related questions. First, how inflammatory signals trigger parietal cell atrophy and second, how inflammatory signals trigger the induction of mucous cell hyperplasia. Related to the first question, her studies have found that the absence of gastrin decreases the levels of parietal cell sonic hedgehog (Shh). Her lab is subsequently testing whether the absence of Shh alters the proliferation profile of the antrum. Related to the second question, she is finding that inflammatory cytokines stimulate the production of mucous and subsequently hypertrophy and hyperplasia of mucous neck cells (mucous gland metaplasia).

### **c. Cell Growth Differentiation and Programmed Cell Death**

#### **i. Peptides and Development**

Recognizing that the mechanisms of adult gastrointestinal disease often reflects mis-regulation of pathways used during embryonic and fetal development, the Peptide Center has continued to foster and grow relationships with basic researchers in gut development. To this end, the Peptide Center has partnered with the Center for Organogenesis in co-sponsoring seminars, and in supporting the inception and maintenance of a Program Project initiative (DK062041, PI: J. Merchant) that is designed to study the mechanisms underlying the cellular decisions of identity in the developing and adult stomach and intestine. Together, four investigators (A. Todisco, L. Samuelson, D. Gumucio and J. Merchant), all Peptide and

Organogenesis Center members, are analyzing molecular events that accompany: intestinal organogenesis, cell lineage decisions in the epithelium (particularly enteroendocrine lineages), development of intestinal metaplasia and differentiation of parietal cells. A pervasive common thread that has emerged from these studies is the importance of hedgehog signaling in several of these events. The Gumucio lab is studying the role of hedgehog signaling in events of intestinal morphogenesis. Recent work in that lab has revealed that hedgehog signals are necessary for the patterning of the crypt/villus axis and the localization of the pre-crypt structures. In the course of this work, the Gumucio lab has developed a number of important tools for the efficient expression of transgenes in the intestinal epithelium. The Samuelson lab is currently using these tools to generate mice models in which NeuroD and other basic HLH molecules are overexpressed in the epithelium; these studies will elucidate the pathways important for enteroendocrine lineage determination. The Todisco laboratory has recently shown that the hedgehog pathway is also involved in the maintenance of parietal cell differentiation; they are further studying the signaling events through which this differentiation process is controlled in a model of isolated canine parietal cells. The Merchant lab is also examining hedgehog signaling in the context of intestinal metaplasia of the stomach. They are investigating alterations in components of the hedgehog pathway (post-translational processing of Shh, distribution of expression of the Patched receptor and Smoothed) during induction of intestinal metaplasia and/or gastric tumors by a variety of mechanisms (bacterial overgrowth, interferon gamma administration, parietal cell ablation by cTox, and the gastrin null mouse model established by the Samuelson lab).

The Morrison laboratory has been studying the mechanisms that regulate stem cell self-renewal, aging, and differentiation. By studying these mechanisms in parallel between stem cells from two different tissues, they assess the extent to which different types of stem cells employ similar or different mechanisms to regulate these critical functions. Specifically, he has studied the genetic regulation of neural crest stem cell function in the developing enteric nervous system. Although he has found no evidence for conserved gene expression profiles among different types of stem cells (Science 301:972), he has identified important mechanisms that regulate the generation, and migration of stem cells in the gut. In particular, we have combined gene expression profiling, with reverse genetics and analyses of stem cell function to show that Hirschsprung's disease (a relatively common birth defect characterized by a failure to form enteric ganglia in the hindgut) is caused by mutations in two pathways that interact to regulate the generation and migration of neural crest stem cells in the gut (Science 301:972, Neuron 40:917). Mutations in the endothelin and GDNF signaling pathways account for the majority of cases of Hirschsprung's disease. We found that these pathways were necessary for the generation of normal numbers of neural crest stem cells in the developing gut, and then for the migration of these cells throughout the gut. Loss of signaling

through either pathway lead to decreases in the number of neural crest stem cells in the gut as well as premature termination of their migration. Aganglionosis appears to be caused by an inability of neural crest stem cells to migrate into the aganglionic portion of the gut. However, this migration defect could be by-passed by transplanting neural crest stem cells directly into the aganglionic region of the gut. These observations raise the possibility that a subset of Hirschsprung's disease patients might be more effectively treated by combining surgery with stem cell transplantation to at least partially restore enteric nervous system function to the affected region of the gut.

The Morrison Lab has developed a technique to prospectively identify and isolate enteric neural crest stem cells (ENCSCs) by flow cytometry. These cells are self-renewing, compose only a few percent of fetal gut cells and can be identified in the proximal gut of a rat model of Hirschsprung disease (HSCR) postnatally and demonstrate neuronal development after injection into the embryonic distal gut of HSCR rats in organ culture. The Garipey and Morrison labs will use these cells to build a distal ENS in individuals with HSCR, with the intent to develop a mechanism to limit the need for surgical resection.

The Liu laboratory is focused on how the nervous system may influence the development and function of different organs. Her primary aim is to understand the genes and cells involved in neuromuscular connections and their potential effects on normal gut ontogeny. She uses the fruit fly as her primary model organism to dissect the genetic pathways and to characterize specific gene functions involved in these developmental processes. Her ultimate goal is to use fundamental information gained from fruit fly ontogeny as portals to gain new insights into the genetic architecture of homologous vertebrate developmental pathways in mice and humans. Presently, she is focused on the function of a family of zinc-finger, homeodomain containing transcription factors, *zfh-1* and *zfh-2*. Both genes are highly expressed in the nervous system, and in the gut, of fruit flies. Null mutation of these genes resulted in nervous system and gastrointestinal defects. Homologues of these fly genes are found in humans and mutation of human *zfh-1* has been implicated in the etiology of a dominant form of Hirschsprung's disease-mental retardation syndrome in humans. She is presently studying the requirement of *Drosophila zfh-1* in normal gut development and how it may participate in the neuronal signaling required for such process. These studies serve as an entry point to further dissect the requirements for neuromuscular innervations in gut development.

## **ii. Cell Growth and Cell Death**

In addition to the role of NOD proteins in innate immunity, the Nuñez laboratory has focused on understanding the signaling pathways involved in apoptosis. A family of cytosolic proteins, termed NODs, has been identified and is being characterized. Two NOD proteins, Nod1 and Nod2, activate NF- $\kappa$ B and appear to be involved in the cellular response to bacterial pathogens. Dr. Nunez is also studying Cryopyrin and Ipaf, two NOD proteins involved in caspase-1 activation and inflammation.

Research in Dr. Colin Duckett's laboratory focuses on the control of cell survival by the iap (inhibitor of apoptosis) 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. One IAP in particular, X-linked IAP (XIAP), is a potent inhibitor of apoptosis that has been shown to play roles in apoptosis inhibition, cell cycle control, activation of stress-induced transcription factors and stress-activated kinases, cytokine signaling, protein degradation through the ubiquitin pathway and even the metabolic control of intracellular copper levels. A major effort in our laboratory is to reconcile these apparently diverse properties of XIAP. Through the characterization of XIAP-deficient mice, mutagenesis analysis, cell imaging studies of intracellular trafficking and the identification of novel XIAP-associated proteins, we are beginning to understand which of these functions can, and cannot, be uncoupled from each other. Our long-term goal is to describe an integrated picture of XIAP in which its disparate functions can be reconciled.

Research in Dr. Ezra Burnstein's lab centers on the regulation of NF- $\kappa$ B, a transcription factor that plays an essential role in inflammation, oncogenesis and cell cycle progression. MURR1 is a recently described regulator of copper homeostasis and deficiency of this factor results in copper toxicity in dogs. In addition, MURR1 is also a potent inhibitor of NF- $\kappa$ B. In a biochemical screen for MURR1-associated factors, the Burstein lab identified a highly conserved family of proteins that share homology to MURR1. All family members possess a unique motif termed the COMM (copper metabolism gene MURR1) domain, which functions as an interface for protein-protein interactions. These proteins are designated as COMMD (COMM Domain containing) 1 through 10. Virtually all human COMMDs associate with NF- $\kappa$ B and suppress  $\kappa$ B-dependent transcription. COMMDs function at the level of transcription initiation to disrupt the association of NF- $\kappa$ B with chromatin. Thus, the COMMDs are a new protein family of NF- $\kappa$ B regulators that exert their effect by controlling the occupancy of NF- $\kappa$ B on chromatin. In addition, Dr. Burstein has recently shown that MURR1 interacts with X-linked Inhibitor of Apoptosis (XIAP), a member of the IAP family. XIAP functions as an E3 ubiquitin ligase for MURR1 through its RING finger domain, resulting in the ubiquitination and proteasomal degradation of MURR1. Consistent with this concept, tissues and cells

derived from XIAP-deficient mice contain higher levels of Murr1 and decreased copper levels, implicating XIAP in the regulation of cellular copper levels through its association with MURR1.

During the course of studying transcriptional regulation of the gastrin promoter, Dr. Merchant and her colleagues cloned a novel factor that termed ZBP-89. This factor was found to repress induction of the gastrin promoter by EGF. Further analysis of this factor has revealed that ZBP-89 represses cell growth by binding directly to the p21<sup>waf1</sup> promoter and stabilizing p53. In addition to growth repression, ZBP-89 triggers apoptosis in a p53-independent manner. Her more recent ongoing studies involve transgenic analysis of ZBP-89 to determine if it is a bona fide tumor suppressor. Biochemical studies using mass spectroscopy revealed that ZBP-89 mediates butyrate regulation to the cell by interacting with another tumor suppressor protein called ataxia telangiectasia (ATM). ATM normally mediates DNA damage signals to the cell. However, her studies suggest that ZBP-89 in collaboration with ATM specifically mediates the butyrate response.