Lung cancer is the nation’s leading cancer killer. Despite continued improvements in lung cancer prevention, such as smoking cessation programs, an estimated 160,000 people died from lung cancer in the U.S. in 2012, which was approximately the same number of deaths as breast, prostate, colon, and pancreatic cancers combined.

Great strides have been made in early detection and targeted therapies over the past 35 years, but so far they have not translated into significant improvements in the 15% 5-year survival rate in patients with lung cancer. This has partly been due to small populations of lung cancer stem cells (CSCs) within the tumor that can cause metastases or repopulate the tumor after the initial treatment. Our group is working to elucidate key signaling pathways driving both lung CSC self-renewal and resistance to chemotherapy or radiotherapy, so that we can sensitize these cells to therapy.

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As the saying goes, Rome wasn’t built in a day. Modern science, too, advances via cumulative, incremental steps over time. Researchers add to the existing body of knowledge in their fields, and together their findings build the foundation for a critical breakthrough.

In health sciences research, “breakthroughs” often result from the accumulation of knowledge over decades. Technology and new interdisciplinary research paradigms are accelerating this discovery process, and simultaneously opening new avenues for knowledge that we could not have imagined 20 years ago.

This issue of UMDNJ Research introduces just a few of our faculty researchers — basic, translational and clinical — who devote their careers to advancing the health sciences. In order for them to do their work, significant funding is required to purchase sophisticated equipment and necessary supplies, underwrite the everyday workings of these labs, and hire and support doctoral and post-doctoral students to conduct research. In 2011, the American Association of Universities reported that universities perform 31 percent of America’s total research — basic and applied — and 56 percent of its basic research.

The National Institutes of Health (NIH) is the leading supporter of biomedical research in the world, and the primary source of research funding at UMDNJ. It is the NIH investment in research at UMDNJ that has made possible many of our important discoveries over the years. In these tight economic times, the NIH, like many other federal agencies, has experienced budget cuts that impact its ability to expand its scientific research portfolio. However, UMDNJ continues to compete effectively for NIH awards, and we expect to continue to build programs and support researchers for future success.

In this issue, we present to you a small segment of our NIH-funded researchers whose work, as you will see, reflects the wide spectrum of UMDNJ’s scientific pursuits, which promise to have major impact on all of our lives.
New findings on the genetic causes of autism and related neurodevelopmental disorders show that many of these conditions stem from disruption of the synapse, the cellular junction through which neurons communicate with each other. In my laboratory we are working on the structure and synaptic function of CASPR2 to address the functional role of this protein in neurons and to gain insights into how it shapes brain development and is implicated in congenital disease.

Autism spectrum disorder (ASD) and autism are both general terms for a group of complex disorders of brain development. In 2012, the Centers for Disease Control and Prevention estimated that one in every 88 children is diagnosed with ASD, making it more common than childhood cancer, juvenile diabetes, and pediatric AIDS combined. Whereas the vast majority of cases of autism are idiopathic, a growing body of genetic studies points to the role of synaptic adhesion proteins in the pathogenesis of some forms of the disease. Most of these genes (e.g., NRXNs, NLGNs, CNTNAP2, CNTNs, RELN, GluD, GluR, NrCAM, IL1RAPL) are important in controlling synaptic function, neuronal connectivity and recognition patterns in the developing brain. Interestingly, some of these genes have also been implicated in the pathogenesis of epilepsy, schizophrenia, ADHD, and Tourette syndrome, suggesting that ASD and these disorders may share common molecular pathways. These psychiatric disorders are commonly associated in the same ASD patient and sometimes family members with the same genetic abnormality have different cognitive disorders. Although CASPR2, encoded by the CNTNAP2 gene, is present in the peripheral nervous system, confined to the juxtaparanodal region of the axon where it associates with the immunoglobulin domains of TAG-1, disruption of the coding region of CASPR2 causes gross histological abnormalities such as cortical dysplasia that are distributed diffusely throughout the human brain.
but not in the periphery. This suggests that CASPR2 plays crucial roles in as yet unidentified mechanisms related to cell-cell interactions important for normal neuronal function and human cortical histogenesis.

In 2006, a paper in *The New England Journal of Medicine* reported the case of nine children belonging to the Old Order Amish families that were homozygous for the rare frame-shift mutation 3709delG (single-base G deletion at nucleotide 3709 in exon 22) in the CNTNAP2 gene. All these kids presented cortical dysplasia, focal epilepsy and autism with severe language regression. A surgical biopsy of the anterior temporal lobe of two children carrying the mutation revealed cortical dysplasia including evidence of abnormalities of neuronal migration, structure of the cortex and widespread migration of astrocytes known as astrogliosis. More recently, 11 rare variants in the same gene were found in children diagnosed with autism and some of their family members. Whereas some of the mutations seem benign, the majority of these variants were predicted to be deleterious because of the type of amino acid change involved and because they occurred in evolutionarily conserved regions of the gene. Although research on the genetic causes of autism is being conducted at an unprecedented pace, very little information is currently available on the molecular and cellular defects resulting from any of these mutations.

In my lab, we have now characterized the subcellular localization of CASPR2 wild type and all point mutations observed in patients to date, and made a detailed analysis of two of these that seem to have the most extreme cellular phenotype (Falivelli et al., 2012, *Human Molecular Genetics*). In particular, CASPR2-D1129H mutant is largely retained in the endoplasmic reticulum (ER) in HEK293 cells and in rat hippocampal neurons. Our results suggest the following overall mechanism underlying the fate of CASPR2-D1129H mutant: the amino acid substitution causes a local misfolding in one of the domains of CASPR2. ER chaperones such as BiP and others remain bound to the mutant, causing the protein to remain in the ER. As BiP is unavailable to suppress unfolded protein response (UPR) activators, the UPR is triggered, the mutant protein is promptly degraded by the proteosomal pathway and its expression is suppressed. The 3709delG mutation results in a prematurely truncated protein that lacks the transmembrane domain. Here, rather than a protein that is ER retained and degraded, our experiments indicate that the 3709delG is promptly secreted by the cell just as efficiently as other natively secreted proteins. These results indicate that the premature stop codon does not interfere with proper domain folding. However, the structural or signaling functions of the membrane tethered form are lost.

To build on these findings, we are now working to link the structure of CASPR2 to its function and therefore address the functional role of CASPR2 at the synapse. Overall, we think that the link between CASPR2 and neurodevelopmental disorders occurs through specific genetic abnormalities (mutations but also deletions and copy number variations), studying the overall structure of the protein and whether these mutants correctly traffic to the cell surface and are normally folded or whether their association with TAG-1 is altered, will help us understand if there is a strong link between CASPR2 mutations, early brain development, and altered social behavior.

During my postdoctoral work at the University of California San Diego, I began working on the structure and function of the synaptic cell adhesion proteins neurelin and neurexin because I had a fascination for synapse biology and I wanted to learn structural biology. At BWJMS, I have been working on a variety of synaptic proteins to understand other aspects of synapse biology. For example, we are engaged in several structural biology projects of other synaptic adhesion proteins and, when available, their binding partners and their complexes. In our lab we mainly use small angle X-ray scattering, single particle electron microscopy, analytical ultracentrifugation, and protein crystallography to solve their three-dimensional structures. Moreover, we are working on the biophysical characterization of the binding of these synaptic adhesion proteins, aiming at understanding protein-protein interaction at the domain, sub-domain, and amino acid level. By solving the structure of a protein, one may be able to develop therapeutic strategies to modify protein biosynthesis, processing or adhesive properties of the partnering proteins to ameliorate the disorder. While this remains on the long-term horizon, the structure of these adhesion proteins, their partnering interactions and their biosynthetic processing become priority targets for understanding the molecular abnormalities underlying ASD and epilepsy.

Davide Comolotti earned his PhD degree from the Mario Negri Institute for Pharmacological Research in Milan, Italy, and did his postdoctoral work at the University of California San Diego. He joined Robert Wood Johnson Medical School in 2011 and works on the structure and function of synaptic cell adhesion proteins linked to autism.

Lab Web site - http://xrjms1.umdnj.edu/comolotti_lab

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**Antimicrobial Peptides — Using Our Natural Defenses to Fight Infections**

by Gill Diamond

The body is constantly in contact with microbes that could potentially cause infection. We breathe in bacteria and viruses that could cause pneumonia or influenza; our mouths are filled with hundreds of species of bacteria, some of which can lead to gum disease and other infections. To prevent these pathogens from taking hold, we have a diverse set of natural, rapid, non-specific first lines of defense, collectively known as innate immunity. These include naturally occurring short proteins with broad-spectrum antibiotic activity, called antimicrobial peptides. My laboratory has been studying these peptides for 20 years, identifying new ones in different species, and trying to understand the role played by some of these peptides in the innate immune defense of two places in the body: the lungs and the mouth. Presently we are taking two different approaches to use these peptides to treat infections. One is to use Vitamin D to enhance the expression of these peptides in their natural settings, and the second is to design small molecules whose structures mimic that of the peptides, and to test them for their ability to treat infections.

**Using vitamin D to boost the peptide-based defense system**

It has been known for a while that people who are deficient in vitamin D have increased respiratory infections, as well as an increase in periodontal disease and the tooth loss that comes with it. Thus, we hypothesized that the active form of vitamin D could lead to an increase in the innate immune defenses in both of these tissues.
Most recently, it was discovered that the production of an antimicrobial peptide called LL-37 could be induced by the active form of vitamin D. This active form, called 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), could increase the expression of the gene that encodes LL-37 in a variety of cells. In collaboration with the laboratory of Dr. Sylvia Christakos of the NJMS Department of Biochemistry, we showed initially that this induction occurred in cultured cells from the trachea and from the gums. In both cases, treatment with physiological concentrations of 1,25(OH)2D3 led to an increase in the expression of the protein as well as an increase in the ability of the cells to kill bacteria that were added to their surface.

We reasoned that this increase in LL-37 might not be the only factor in the vitamin D-mediated defense. Using a PCR array technique, we looked for other genes involved in innate immunity that were induced by vitamin D. We found in both cases that several other genes were indeed induced. These included CD14, which is a receptor for the lipopolysaccharide molecules found on the outside of some bacteria, and Triggering Receptor Expressed on Myeloid Cells (TREM)-1. This latter receptor is also involved in regulating the cells’ response to pathogens as part of the innate immune defense. When gingival cells were stimulated with both 1,25(OH)2D3 and an antibody that binds to TREM-1 and activates it, the cells exhibited even more antibacterial activity than with 1,25(OH)2D3 alone, suggesting that the vitamin D was increasing several aspects of the innate immune defenses in these cells.

Our next step is to take this observation into a mouse model, where we can examine the potential for vitamin D as a therapeutic agent either to prevent or treat both respiratory tract infections and the gum disease that results from bacterial growth in the gingival crevice. Unfortunately, the mouse gene that is homologous to the human LL-37 is not regulated by vitamin D. With help from a collaborator, Dr. Adrian Gombart at the Oregon Health Sciences University, we will be using a transgenic mouse strain that expresses the human LL-37 gene, under the control of its own regulatory elements, and which is induced by vitamin D. Our goal is to understand how vitamin D can regulate the innate immune defenses against respiratory and oral infections, and how it might be used to treat or prevent those infections.

Turning peptides into drugs

When the first antimicrobial peptides were discovered, initially in such diverse places as human white blood cells and frog skin secretions, they were shown to be potent, broad-spectrum antimicrobial agents. More importantly, microbes did not appear to develop resistance to them, suggesting that these peptides could become the next generation of antibiotics, helping to solve the problem of increasing resistance to antibiotics leading to such life-threatening infections as methicillin-resistant Staphylococcus aureus (MRSA). Unfortunately, once pharmaceutical companies attempted to develop these peptides into usable antibiotic drugs, they did not work in a clinical setting. This appeared to be due, in large part, to problems associated with the fact that peptides are short proteins, and can be digested as such. One way to address this problem was developed by a group at the University of Pennsylvania, where they studied the structure of the peptides, and designed a series of small molecules that mimic the antimicrobial portion of the peptide. These small molecules, called peptide mimetics, exhibit similar antimicrobial activity against the pathogens, but are not peptides and thus might be more active when used as drugs.

We have been working with a small biotech company that was started to develop this peptide mimetic technology, called PolyMedix, Inc. Together with their Vice President for Research, Dr. Richard Scott, we have begun to examine the potential of these mimetics for treating fungal infections. We started by screening different mimetic structures for their activity against the common fungus, Candida albicans, the microbe that causes oral candidiasis (also known as thrush), as well as fatal blood infections. To be sure that the drug was specific for fungus, and did not harm the normal flora in the mouth, we identified mimetics that were also inactive against two species of bacteria commonly found in the mouth. When cultures of Candida are treated with the mimetic, it leads to a rapid lysis of the cells, as we can see by the influx and efflux of small molecules, as well as by the visualization of bursting of the cells by fluorescence microscopy. We then demonstrated that these lead compounds exhibited potent antifungal activity against the hyphal biofilm form of Candida that is found in oral infections, and that they were active in the presence of saliva. All of this information supported our hypothesis that the drugs could be used to treat oral fungal infections. Finally, we tested the drugs on two different mouse models of oral candidiasis. In both cases, single administration of the drugs was sufficient to almost completely ablate the infection. As a result, together with PolyMedix, Inc., we have initiated the process to test our best compound in human infections.

Since their discovery in the 1980s, scientists have been trying to figure out how to harness these naturally occurring antimicrobial peptides, and to use them as drugs. We are hoping that the two complementary paths taken in my laboratory will lead to useful antimicrobial therapies.

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*Gill Diamond received his PhD in genetics from the Hebrew University of Jerusalem, and did a postdoctoral fellowship at the Children’s Hospital of Philadelphia before coming to UMDNJ in 1993. He has been the director of the PhD program in Biomedical Sciences since 2002. Since coming to the University, he has studied antimicrobial peptides, examining their role in innate immune defense of the airway and the oral cavity, as well as the potential for their development as therapeutic agents for microbial infections.*
Often suffering with our own headaches from exposure to high altitude and air pollution, we wondered — while looking through the microscope in our Mexico City collaborator’s lab — how cells from the bronchoalveolar spaces of the human lungs that we studied would be able to fend off inhaled bacteria while being loaded with the brownish-black-appearing inhaled particles we observed.

The lungs are the primary portal of entry for aerosolized fine particulate matter (PM2.5, PM with aerodynamic diameter less than 2.5 µm) and *Mycobacterium tuberculosis* (*M.tb*), the bacterium that causes tuberculosis (TB). Globally, TB continues to be a public health emergency as declared by the World Health Organization (WHO) and remains one of the world’s major causes of illness and death.

Our studies of lung immunity during the past two decades had provided us with insights into the functional capabilities and responsiveness of alveolar macrophages and lymphocytes that are present along the inner walls of the alveolar spaces, where gas exchange takes place during TB and in health. One of the multiple functions of alveolar macrophages is to take up foreign matter, whether in the form of bacteria, fungal spores or airborne particles, which reach the lower airways due to their small size and physicochemical characteristics.

With our interest in human lung immune defenses during TB, the presence of these particles in cells from Mexico City dwellers encouraged us to develop a mechanistic model that would help in the assessment of the particles’ effects on antibacterial immune cell functions. Diesel exhaust particles (a major component of urban air pollutants) obtained in collaboration with investigators at the Environmental and Occupational Health Sciences Institute, a shared UMDNJ-Rutgers program, became our initial study material. How would exposure of human immune cells to these particles alter the cells’ ability to respond to *M.tb*? This was not just an academic question.

There is now indisputable epidemiologic evidence that air pollution and TB are associated. We hypothesized that PM2.5 could promote TB susceptibility in Mexico City inhabitants. To test this hypothesis, we decided to focus on the innate immune system, which is the body’s first line of defense against pathogens.

In our research, we observed that exposure to PM2.5 increased neutrophil chemotaxis, which is crucial for the mobilization of immune cells to infection sites. This effect was associated with an increase in the production of pro-inflammatory cytokines, such as interleukin-8 (IL-8), which plays a key role in recruiting neutrophils to the site of infection.

We also found that PM2.5 exposure enhanced the phagocytic activity of alveolar macrophages, which are critical in the removal of inhaled particles and the clearance of pathogens. This enhancement was accompanied by an increase in the production of reactive oxygen species (ROS), which are potent antimicrobial agents.

In conclusion, our research suggests that air pollution, particularly PM2.5, can have significant implications for the susceptibility to and progression of TB. These findings highlight the need for further research to understand the complex interactions between air pollution and the host immune system, and to develop strategies to mitigate the health effects of air pollution on TB control and prevention.
pollution exposure globally increases the risk of respiratory conditions such as asthma and infections, as well as cardiovascular disease and diabetes mellitus. Air pollution affects everybody worldwide; only the type, extent, time and location of the exposure vary. As one of the reviewers of my grants stated, “No one who has traveled extensively through the large cities in the developing world can have failed to notice the deteriorating air quality resulting from rapid industrial growth. Any primary care physician can attest to the enormous increase in respiratory disease in these environments.”

More specifically there is overwhelming epidemiologic evidence that exposure to tobacco smoke, indoor air pollution from combustion of fossil fuels in the process of cooking, or occupational exposure to silica significantly increases the risk of developing TB. Air pollution and TB each contribute significantly to global disease burden as deteriorating air quality from rapid, unregulated industrial growth and traffic collide with high levels of endemic TB in many parts of the world. Indeed, with more than half of humankind living in urban settings, indoor and urban outdoor air pollution rank 10th and 14th respectively among 19 leading risk factors for global mortality according to WHO.

Initial studies in our lab showed that exposure of human blood cells to diesel particles altered the cells’ ability to respond to M.tb. We assessed the expression of groups of gene targets (mRNA) and proteins that participate in protective antimycobacterial host responses. We found strong evidence that signaling mechanisms originating from cellular receptors that co-orchestrate innate immune responses (toll-like receptors) were altered when cells had been exposed to the particles. More specifically several NF-κB and IRF-1-mediated target genes were suppressed that are required for appropriate antimycobacterial immunity. These responses were dose-dependent and, interestingly, were particularly extensive in cases when the cells had been exposed to the particles prior to Mtb exposure. Similar findings were seen when we looked at the expression of inflammatory cytokines and chemokines.

With this important finding published in the Journal of Immunology in 2012, we assembled a team of researchers from Mexico City, University of Alberta in Canada, University of Southern California, and University of Michigan, and from the departments of Environmental and Occupational Health and Biostatistics at the UMDNJ School of Public Health (SPH) in Piscataway. We applied for NIH support to perform a multi-institutional, international, translational research project. This resulted in the recent award of a five-year RO1 grant to examine the role of matroy cytokines, innate and adaptive antimycobacterial immune effector functions, and the uptake and growth control of M.tb by these cells. Our studies will also try to answer the question whether the composition and seasonal variations of the air pollution particles may not well understood.

With growing air pollution worldwide, we hope that the timeliness and urgency of our research questions will generate new knowledge with significant impact on global public health and policy.
Duchenne Muscular Dystrophy (DMD) is an inherited lethal muscular disease that primarily affects adolescent males. It is usually diagnosed in early childhood. For a long time it was considered to be predominantly a skeletal muscle illness. Clinically, it was associated with progressive debilitating muscle weakness, skeletal deformities and breathing disorders. Cardiac complications of this disease became prominent relatively recently as the life of DMD patients could be prolonged with improved therapies for skeletal muscle, including assisted ventilation and corticosteroid treatment. Most of the patients with DMD develop dilated cardiomyopathy and arrhythmia by 20 years of age. Cardiac complications limit survival of a significant number of these patients. Further prolongation of life and amelioration in the quality of life for DMD patients depends not only on improving skeletal muscle performance but also on the development of therapeutics that slow down the progression of the cardiac disease and enhance cardiac function. This requires a mechanistic understanding of the nature of the cardiac defects. Therefore, we are focusing our current research on the studies of the cellular and molecular mechanisms leading to dystrophic cardiomyopathy.

There are several experimental animal models of DMD. We employ mdx mice that have a genetic defect similar to DMD — lack of the functional protein dystrophin. The dystrophin network covers almost the entire inner surface of cellular membrane and helps to maintain mechanical stability of skeletal and cardiac myocytes. It is widely accepted that the predominant functional consequence of the absence of dystrophin is an increased membrane vulnerability to mechanical stress associated with muscle contraction. However, a growing body of literature reveals that several pathomech-

Stress-induced calcium wave in a dystrophic cardiomyocyte

Looking Inside Dystrophic Hearts

by Natalia Shirokova
and to identify the potential molecular targets for early intervention with the disease. In our work we mostly concentrate on two proteins, which we believe to play crucial roles in the early progression of dystrophy. They are NADPH oxidase and RyR.

NADPH oxidase type 2 (nicotinamide adenine dinucleotide phosphate-oxidase, NOX2) is a membrane-bound protein complex and one of the main sources of production of reactive oxygen species (ROS) in the cardiovascular system. As stated above, our results obtained in cardiomyocytes isolated from young mdx mice suggest that in these cells ROS production was already abnormally high. Furthermore, ROS scavengers and inhibitors of NADPH oxidase could normalize ROS levels and prevent most of the acute Ca2+ signaling alterations, suggesting NOX2 as the primary source of this oxidative stress. Why and how NOX2 becomes abnormally activated in dystrophic cells is not entirely clear. Overexpression of the enzyme and/or an increase in its activity through several not-exclusive cellular pathways are currently being investigated.

NOX/ROS mediated signaling has a significant downstream impact on Ca2+ signaling proteins including, but not limited to, RyR receptor (ryanodine receptor, Ca2+ release channel of the sarcoplasmic reticulum (SR)). Several groups, including ours, demonstrated that at different stages of dystrophic cardiomyopathy, its cellular phenotype exhibits abnormally augmented intracellular Ca2+ responses (i.e. a burst of Ca2+ sparks and waves) to mechanical stress, hypersensitive excitation-contraction coupling, increased Ca2+ leak from the SR and reduced SR Ca2+ load. These features clearly point to modifications in intracellular Ca2+ cycling and suggest elevated activity of Ca2+-induced Ca2+ release (CICR) from the SR via RyR. Moreover, our recent findings showed that these features are present in the hearts of very young dystrophic animals. As underlying causes, several and probably not mutually exclusive mechanisms of RyRs sensitization have been proposed. They include oxidative and nitrosative post-translational modifications of RyRs, increased Ca2+ leak from the SR due to oxidative stress, increased RyR phosphorylation by PKA or CaMKII. However, causal/adaptive relationships between these modifications and alterations of RyR function, development of cellular abnormalities and progression of dystrophic cardiomyopathy were not established clearly.

One possible strategy to gain insight into the interplay between causal and adaptive alterations of cardiac muscle is to follow the disease progression over longer periods of time. This approach is based on the rationale that causal changes can presumably be identified from the beginning of the disease, even before the pathology becomes manifest, while adaptive beginning of the disease, even before the changes can presumably be identified from the approach is based on the rationale that causal alterations of cardiac muscle is to follow the disease interplay between causal and adaptive alterations, suggesting NOX2 as the primary source of this oxidative stress. Why and how NOX2 becomes abnormally activated in dystrophic cells is not entirely clear. Overexpression of the enzyme and/or an increase in its activity through several not-exclusive cellular pathways are currently being investigated.

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Our studies also suggest that later, during the progression of dystrophic cardiomyopathy, but before heart failure is observed, nitrosation and PKA-dependent phosphorylation further contribute to the increased sensitivity of RyRs to Ca2+. At this stage of the disease mdx mice as well as DMD patients often exhibit electrocardiographic abnormalities, such as premature ventricular beats. Moreover, during physical or emotional stress CPVTs (Catecholaminergic Polymorphic Ventricular Tachycardias) have been reported in boys with DMDs. It is possible that under these conditions β-adrenergic input progressively accumulating mechanical damage to the cells, but also from initially adaptive and later maladaptive remodeling of the cardiac muscle cells and tissue. Crosstalk between several of the known pathomechanisms and Ca2+ signaling pathways may be instrumental for the time-dependent evolution of the disease phenotype. If a similar scenario leads to dystrophic cardiomyopathy in human patients, this complexity might have repercussions for the development of novel therapeutic approaches.

Finally, our data indicate that at the terminal stages of cardiac dystrophy, several post-translational modifications together hypersensitize RyR to an extent where the SR Ca2+ leak dramatically increases. This may subsequently contribute to cytosolic and mitochondrial Ca2+ overload, activation of necrotic and apoptotic processes and loss of functional myocytes. The latter step usually precedes the development of cardiac fibrosis and reduction in heart contractility. In parallel, enhanced and uncompensated SR Ca2+ leak results in a reduction in SR Ca2+ load, which in turn reduce the amplitude of beat-to-beat intracellular Ca2+ transients, thus resulting in reduced force production by the surviving cardiomyocytes. These two cellular processes could eventually impede cardiac contractility leading to cardiac failure.

Overall, there are a number of main conclusions that can be drawn from the studies of several laboratories around the world (groups of Drs. J. Metzger, E. Neglia, D. Allen, B. Petroff, A. Marks, J. Lederer, X. Wehrens and several others). It is becoming clearer that multiple pathomechanisms are involved in the cellular damage initiated by the lack of dystrophin, in both skeletal and cardiac muscle. During the slow progression of the cardiomyopathy, the activation of these damaging cellular pathways is orchestrated in an ever changing pattern and severity. This may arise from

At present, there are several ongoing preclinical and clinical trials (e.g. treatment with antioxidants, PDE inhibitors, ACE and Ang II receptor inhibitors, β-blockers, membrane sealants and mitochondrial stabilizers). Each trial is targeting specific mechanisms thought to be of key importance for muscular dystrophy. However, it might be more prudent to apply combinational therapies targeting multiple cellular pathologies simultaneously, and to adapt the treatment regime to the time-dependent prevalence of each of the pathways.

Natalia Shirokova, a native of the Ukraine, received an MS degree in chemical and molecular physics from the Moscow Institute of Physics and Technology in Russia, and a PhD in physiology and biophysics from Bogomoletz Institute of Physiology in Kiev. She carried out postdoctoral research at Rush University, Chicago, and joined the faculty of New Jersey Medical School in 2000. Dr. Shirokova has received continuous funding from multiple grant agencies, such as the NIH, AHA, MDA and Swiss Muscle Foundation for the last 15 years. Her research focuses on skeletal and cardiac muscle physiology and pathophysiology. Recent research efforts of this laboratory have concentrated on molecular and cellular mechanisms underlying the development of dystrophic cardiomyopathy.
or decades, the tobacco industry knowingly deceived American consumers about the health risks of smoking, manipulated its products to make them more addictive, exploited regulatory loopholes, and engaged in highly successful but misleading marketing practices. On June 22, 2009, President Barack Obama signed the Family Smoking Prevention and Tobacco Control Act (TCA) into law and dramatically altered the tobacco control landscape, fundamentally changing how the industry does business. Under the new provisions, the Food and Drug Administration [FDA] is tasked with regulating the production, sale and marketing of tobacco products. Perhaps the most visible change will be improvements to the warning labels on cigarettes and smokeless tobacco products. But the FDA’s powers are broad. For example, the FDA can require tobacco manufacturers to make product changes that are “appropriate for the protection of the public health,” such as the reduction or elimination of harmful substances, like arsenic, which is added during the cigarette manufacturing process. Limitations do exist on FDA regulatory authority. They cannot ban tobacco products nor reduce nicotine yields to zero. In addition, current regulations only apply to cigarettes and smokeless tobacco, but the FDA signaled that it intends to assert its authority over cigars and electronic or e-cigarettes soon.

Following the passage of the TCA, the FDA partnered with the National Institutes of Health [NIH] to foster tobacco control regulatory science. Priority research areas include understanding the diversity of tobacco products,
communication about tobacco products, and tobacco product marketing. Researchers at our Center for Tobacco Surveillance and Evaluation Research at the School of Public Health currently conduct research that is highly relevant to tobacco product regulation and continue to search for new ways to evaluate and inform FDA regulatory authority. Our research agenda is focused on exploring the changing product features of the current tobacco marketplace, shifts in tobacco industry marketing techniques, and tobacco-related behaviors and attitudes among various sub-populations.

Facing declining cigarette sales, the tobacco industry has promoted non-cigarette tobacco products, such as smokeless tobacco and cigars, as well as new products, like the e-cigarette. Within these product categories, manufacturers have promoted features, such as flavors, that were banned for cigarettes under the TCA. Supported by various NIH grants since 2005, we have assessed the non-cigarette tobacco marketplace using convenience store, data gathered from regional and national growth trends. Our ongoing research finds that consumption of these products is steadily increasing, in contrast to cigarette consumption. Moreover, product features like flavors for cigars and smokeless tobacco and "portion pouches" for smokeless tobacco are driving growth. Increased consumption of smokeless tobacco may be attributable to new users (e.g., youth and/or smokers trying to quit or cut back), the ease of initiation associated with flavors and "portion pouches" certain brands. Our team is currently fielding a national survey of current tobacco users to assess patterns of smokeless tobacco use and perceptions of various brands. As cigarette prices continue to increase and smoke-free air laws restrict smoking in more public places, we expect smokeless tobacco sales to trend upward, perhaps due in part to smokers looking for a cheaper and indoor alternative to cigarettes.

The 1998 Master Settlement Agreement between the tobacco industry and 46 states put an end to specific egregious marketing tactics, such as the use of cartoons in advertisements and billboard displays, to protect youth. Subsequently, young adults are the youngest legal targets of the tobacco industry and currently display the highest rates of cigarette use, and notably higher rates of use of non-cigarette tobacco products such as cigars and smokeless tobacco. To this effect, we recently completed a nationally-representative survey of 2,700 young adults, ages 18 to 34. Using this cohort, we investigated tobacco-related behaviors and perceptions about "reduced risk" products like e-cigarettes. Under the new TCA, tobacco companies will be able to make explicit health-related claims in marketing of certain products if the FDA approves them as "modified risk tobacco products" (MRTPs). As such, assessing perceptions of potential MRTPs among young adults might reveal important information about the extent to which these products are adopted and the manner in which they may be used. Preliminary data reveal that awareness of e-cigarettes is high (75%) and nearly a third of the current young adult smokers have tried them. Moreover, two-thirds of smokers believe that e-cigarettes are less risky than cigarettes. This data, along with a second wave of data collection that will occur later this year, will provide critical data on tobacco use behaviors, attitudes, and beliefs about emerging tobacco products and can inform regulatory decision-making regarding these products.

Faced with increased marketing restrictions, tobacco companies shifted their advertising to "under the radar" marketing tactics such as direct mailings to consumers aimed at increasing brand loyalty and continued product use. Since 2002, under the leadership of Dr. Jane Lewis, the Center has maintained an online database called "Trinkets and Trash" (www.trinketsandtrash.org) that monitors, collects and documents industry marketing materials including direct mail pieces (see Figure 1). The publically available archive has informed a number of NIH-supported research projects. We are now fielding an online survey to tobacco users that will provide the first national data on prevalence of receipt of direct mail/email, investigate the influence on smoking behaviors of direct mail and the incentives and rewards for smoking it carries, and assess public opinion about policies intended to restrict this type of marketing. Review of direct mail pieces in our collection shows that many are currently sent in colorful envelopes carrying images of tobacco products. Anyone living in the household, including children, may be exposed to (and therefore influenced by) this imagery, which runs counter to tobacco control's efforts to limit youth exposure to tobacco marketing. Any effort to address this or otherwise restrict industry marketing, however, will certainly be met with opposition from tobacco companies. Indeed, in 2011, in response to an attempt by the FDA to place graphic warning labels on cigarette packs, tobacco companies sued the FDA, claiming that the new labels violate their first amendment rights. The results of our direct mail research, along with our continued surveillance of tobacco marketing, will help inform policy related to tobacco industry advertising.

Our NIH-supported research agenda documents important changes in the tobacco marketplace as a function of regulation and industry marketing, as well as assessing the impact on behavior. Future research will investigate factors, such as packaging and coupons, which may be driving tobacco use, and will assess risk perceptions of products that make implicit reduced risk claims.

The tobacco industry, in opposition to sweeping tobacco control reform, is fighting back with product manipulation, innovative products, and targeted advertising to retain their current consumers and acquire new users. The recent partnership between FDA and NIH presents a unique opportunity for regulatory science research, and our Center is well-positioned to conduct and disseminate the needed science to inform effective tobacco control regulation and protect public health.

Cristine Delnevo has spearheaded efforts to use unique methods (such as cell phone RDD sampling) and data sources (such as market scanner data) to enhance tobacco control surveillance. She earned a PhD from Temple University and a MPH from The New Jersey Graduate Program in Public Health, a collaboration of UMDNJ’s Robert Wood Johnson Medical School and Rutgers’ Bloustein School of Planning and Public Policy. She has been at UMDNJ since 1989.

CRISTINE DELNEVO, PHD, MPH, DIRECTOR, CENTER FOR TOBACCO SURVEILLANCE AND EVALUATION RESEARCH AND PROFESSOR AND CHAIR, DEPARTMENT OF HEALTH EDUCATION/BEHAVIORAL SCIENCE, SCHOOL OF PUBLIC HEALTH
Calcium, Clocks and Malaria

by Andrew P. Thomas

In the fictional movie Jurassic Park, dinosaur DNA is isolated from ancient dinosaur blood preserved in the gut of mosquitoes embedded in amber millions of years ago. That blood may also have contained malaria parasites. This ancient pathogen has been put forward as a possible contributor to the extinction of dinosaurs, and its complex life cycle of transmission between a mosquito vector and vertebrate host has proven to be a remarkably successful evolutionary adaptation.

There are more than 200 species of malaria parasites, members of the protist genus Plasmodia. Each of the Plasmodia species is adapted to infect a specific vertebrate host, and these hosts include reptiles, birds and mammals. Several species of Plasmodia infect humans, including the most deadly form, Plasmodium falciparum. In addition to the highly evolved utilization of the insect vector for transmission, another host with a second mosquito bite. But perhaps its most surprising adaptation is our discovery that this single-celled intracellular parasite has the machinery to respond to endocrine signals that regulate circadian rhythm, synchronizing its life cycle with the body clock of its human host.

Malaria is endemic in many parts of the developing world, including much of sub-Saharan Africa, and parts of India, South East Asia and South America. The CDC estimated a total of 216 million cases worldwide in 2010, with more than 650,000 fatalities. Deaths from malaria...
There are more than 200 species of malaria parasites, members of the protist genus *Plasmodia*. Each of the *Plasmodia* species is adapted to infect a specific vertebrate host, and these hosts include reptiles, birds and mammals, occurring predominantly in young children and pregnant women. The malaria parasite is carried by the female Anopheles mosquito, which injects *Plasmodium* sporozoites into the host while taking a blood meal. These parasites first invade liver hepatocytes where they go through a replicative stage parasites back to the blood. During the liver phase the disease is generally asymptomatic and the number of parasites relatively low. But in the subsequent red blood cell phase the number of infected erythrocytes may reach a trillion (5% parasitemia), through a series of cycles of red cell invasion, parasite proliferation, red cell lysis and reinvasion of uninfected cells. During this erythrocyte cycle the parasites grow and replicate to release up to 24 new merozoites over a period of 2-3 days. Eventually, some parasites develop into gametocytes and are taken up during feeding by another mosquito, initiating the vector phase of the life cycle which generates new sporozoites to be injected into the next host.

The symptoms of malaria are caused by the destruction and physical alteration of red blood cells together with the associated immune response, and include fever, nausea, anemia and hemoglobinuria. In severe cases this can lead to renal failure, cardiovascular collapse and cerebral malaria, where infected red cells adhere to the microvasculature and block blood flow in the brain. A remarkable feature of malaria is that the fever and associated symptoms often occur periodically, repeating in multiples of 24 hours, either every two (tertian fever) or three (quartan fever) days. For *P. falciparum* the cycle repeats every 48 hours. This periodicity reflects synchronization of the erythrocytic cycle, such that red blood cell lysis and parasite release occur synchronously.

The unique pattern of synchronized proliferation allows the malaria parasites to be sequestered for most of the growth cycle, with brief surges of exposure during synchronized red cell lysis that can overwhelm the host immune system prior to reinvasion and sequestration. This may contribute to the fact that many individuals undergo multiple bouts of malaria without developing effective immunity, and could be a factor in the poor performance of malaria vaccination strategies. It might be argued that synchronization of the erythrocytic cycle occurs because the parasites derive from a single mosquito bite that initiates their intrinsic cell cycle clocks. However, the parasites can actually become more apparent with increasing cycles as the disease progresses in untreated patients. Moreover, in a mouse malaria model it has been shown that simply inverting the timing of the day-light cycle can reset the parasite cell cycle clock to match. So this raises two questions: how do the malaria parasites know what time of day it is, and how can billions of parasites remain synchronized to the host circadian rhythm sequestered inside red blood cells?

My interest in malaria started with a collaboration in which we used confocal microscopy to study trafficking pathways between the intraerythrocytic parasite and the red blood cell surface. That study, published in *Nature* in 1991, initiated a long fascination with the cell biology of *Plasmodia*. We went on to collaborate with Dr. Celia Garcia of the University of Sao Paulo in Brazil, applying our expertise with live cell fluorescence imaging to study the cell physiology of erythrocyte-stage parasites. Nevertheless, the primary focus of my laboratory is not on parasitology, immunology or infections disease, but on the endocrine control of calcium signaling in liver and heart. We are interested in the mechanisms by which oscillatory calcium signals are decoded to regulate metabolism and mitochondrial function. In particular, our studies over the last 25 years have defined basic properties of the calcium mobilizing second messenger inositol 1,4,5-trisphosphate (IP3) and its role in generating complex temporal and spatial intracellular signals.

Our work on calcium signaling gave us a unique window into how malaria parasites remain synchronized during the erythrocytic cell cycle. With Dr. Garcia, we demonstrated that IP3 can release calcium from intracellular organelles in malaria parasites, and that these organisms maintain calcium stores and homeostatic mechanisms even while sequestered within the host erythrocyte. However, the breakthrough in understanding how the malaria clock is synchronized came with the discovery that parasite calcium signals can be elicited by the human host hormone melatonin. Melatonin secretion from the pineal gland is suppressed during daylight but surges at night, providing an endocrine component of the mammalian circadian rhythm or body clock. And it is this host endocrine signal that certain Plasmodia species have apparently evolved to detect.

Melatonin is relatively lipophilic and can pass through erythrocyte membranes to act on intracellular parasites. This can be shown by the transient calcium increases induced by melatonin, which occur specifically in the parasite cytosol within the host red blood cell. Melatonin stimulates phospholipase C activity and the generation of inositol phosphates in the parasite, analogous to the IP3-dependent calcium signaling pathway in mammalian cells. Moreover, we have shown that photorelease of IP3 from caged-IP3 loaded into the parasite induces a similar calcium signal to that caused by melatonin. Significantly, in the mouse malaria model, elimination of the host pineal gland or treatment with a melatonin receptor antagonist can desynchronize the parasite lifecycle and reduce parasite proliferation. These findings have led us to postulate that there may be a unique malarial melatonin receptor, which is responsible for entraining the proliferation cycle of the parasites to the circadian rhythm of the host.

It is this hypothesis that forms the basis of our recent NIH grant to investigate the *Plasmodium* melatonin receptor as a therapeutic target. With Dr. Garcia and Drs. Paula Bartlett and Joel Freundlich in the Department of Pharmacology & Physiology, we are working to elucidate the malarial melatonin signaling pathway and identify the *Plasmodium* melatonin receptor. We already have some drug leads that appear to target this pathway. Our goal is to disrupt the way in which malaria locks on to the host body clock. By blocking the malarial melatonin receptor we expect to interfere with parasite proliferation and desynchronize the *Plasmodium* life cycle, making it more susceptible to elimination by the immune system. This would represent a unique therapeutic paradigm, targeting this uniquely adapted parasite.

Andrew P. Thomas is the holder of the Thomas P. Infusino Endowed Chair at UMDNJ-New Jersey Medical School. He obtained his PhD from the University of Bristol, UK, and carried out postdoctoral training at the University of Pennsylvania. He came to UMDNJ from Thomas Jefferson University in 1997. Internationally recognized for his work on IP3-dependent calcium signaling and regulation of mitochondrial metabolism, he has 25 years of continuous NIH funding and more than 150 publications.
Making New Neurons: Stimulating for Development & Deficient in Disease

By Emmanuel DiCicco-Bloom

With the discovery that we make new neurons throughout life, we have been able to learn the basic principles that regulate this process, and investigate how changes in these pathways contribute to human disease. By isolating precursor cells from different brain regions, we have found that growth factors can stimulate or inhibit cell proliferation by controlling several components of the cell cycle machinery. As a result, the brain regions can be too large with too many neurons, as observed in autism, or too small, as seen in schizophrenia and depression. These fundamental insights are critical to understanding how autism associated genes affect brain development, and re-creating human neurons from stem cells to define the neurobiological signature of the disease.

This is a pair of embryonic mouse brains viewed from above. The one on the left (WT) is the normal size and shape. The one on the right (KO) comes from an embryo missing the gene for cell proliferation inhibitor, p57Kip2, and is quite enlarged, especially in the cerebral cortex (at top).

When I completed training in child neurology at New York Hospital-Cornell Medical Center, we believed and told patients that we would ever have, and will lose 10,000 cells each day! So much has changed in the intervening three decades. After discovering we could make new neurons (neurogenesis) in a culture dish, and then in living animals, we began imagining the possibilities of addressing brain diseases using newly generated cells. Indeed, now there are legions of researchers, in basic laboratories to neurology clinics, who are manipulating neural stem cells to define their roles in disease and employ them as therapeutic tools.

Where We Began
In our laboratory, we have used pure populations of neuronal precursor cells (stem cells) to explore how growth factors, like IGF1, FGF and neurotrophins (NGF, NT3, BDNF), regulate proliferation and survival. To determine whether
Development of the Cerebral Cortex

Some of our most interesting research has focused on the cerebral cortex, which is responsible for our most sophisticated abilities like language, emotions, judgment, and movement. While different cortical regions mediate different capacities, they all exhibit a common tissue architecture comprised of six layers. Interestingly, deep cortical layers are generated first during gestation, whereas upper layers are produced later. Which signals determine when a precursor cell stops dividing? How do they work? And do changes in these pathways contribute to neuropsychiatric diseases? Working in culture and in the developing fetus in utero, we found that cortical stem cell proliferation and neurogenesis are regulated by a balance of positive and negative signals. Competing stimulatory and inhibitory signals are well known to underlie cancer proliferation. In cortical stem cells, we found that extracellular signals can stimulate (IGF1, FGF) or inhibit (PACAP) proliferation, but activity may be stage dependent. Furthermore, individual signals modulate both limbs of the cell cycle machinery, altering activators of proliferation, the cyclins, as well as inhibitors, p57Kip2 and p27Kip1, through transcriptional and posttranscriptional mechanisms. Moreover, p57Kip2 plays a key role, controlling the cell cycle timing and also determining cell fate by regulating the numbers of divisions and types of neurons produced. These basic studies are relevant to human diseases: changes in IGF1 are implicated in macrocephaly and autism; FGF signaling is dysregulated in depression; PACAP dysfunction contributes to schizophrenia, PTSD and autism; and p57Kip2 underlies overgrowth in Beckwith-Wiedemann syndrome.

Environmental Factors Disrupt Neurodevelopment

Building on this foundation, we are now defining the effects of environmental toxins, like methylmercury (MeHg) and dioxin, and the therapeutic drug, valproic acid (Depakene), on developmental neurogenesis. In child neurology, families frequently raise concerns about children’s problems with learning and memory, and wonder whether environmental pollutants are involved. We all have measurable blood levels of MeHg that we obtain from our diet, especially fish and seafood. A major brain region involved in learning and memory, which is reduced in depression and schizophrenia, is the hippocampus. Interestingly, the formation of new memories involves neurogenesis in the hippocampus. New neurons are produced every day, and positive and negative experiences, including exercise, diet, stress, drugs, radiation and disease, influence how many cells are made and whether they live or die. In a series of studies in newborn rats, a period comparable to the third trimester of gestation, we have found that very low levels of MeHg selective-ly kill hippocampal stem cells. These deficits persist into adolescence, where rats have decreased hippocampal neurons and most importantly, impaired learning and memory. Our studies suggest that dietary MeHg contributes to developmental learning problems, and support further restrictions on fish consumption during pregnancy. Our recent studies suggest MeHg toxicity can be prevented by administering N-acetylcysteine, an antioxidant used for acetaminophen poisoning. Current studies are refining these treatments and determining the period of development MeHg vulnerability. This same paradigm has defined negative effects of dioxin, the Vietnam war defoliant, as related polychlorinated biphenyls (PCBs) remain persistent pollutants.

Drugs, Genes and Autism

The anticonvulsant, valproic acid (VPA), is used therapeutically to treat seizures, prevent migraine and stabilize mood. While long known to cause congenital malformations, recent evidence indicates that children exposed in utero may exhibit behaviors related to autism and mental retardation. In cancer research, VPA is a well known inhibitor of proliferation, and new pharmacotherapies are being developed. Surprisingly then, and contrary to expectations, we found VPA stimulates cortical stem cell proliferation by acting via the cell cycle. VPA treatment increased cortical neurogenesis, especially in upper layers, and produced macrocephaly. Since VPA is an autism risk factor, it is interesting that it produced macrocephaly, because ~20% of people with autism have enlarged brains. Two other projects also focus on autism, in collaboration with James Millotig. First, following his discovery that histone patterning gene, Engrailed2, is associated with autism, we are defining effects of gene deletion. We find not only hindbrain deficits, but also unexpected changes in forebrain, including reduced monoamine neurotransmitters, increased hippocampal neurogenesis and abnormal autism related behaviors such as social interactions and cognition. Second, we are producing induced pluripotent stem cells from people in New Jersey who have autism to identify the neurobiological signature of their disorder, and design specific therapies.
Vitamin D is critical for optimal calcium homeostasis and bone health. In addition, recent evidence indicates the involvement of vitamin D in diverse cellular processes including inhibition of cancer progression, modulation of innate immunity with subsequent killing of bacteria and inhibition of certain autoimmune diseases. Studies related to the mechanism of vitamin D action have provided new insight into both skeletal and non-skeletal actions of vitamin D that may suggest new therapeutic targets.

Vitamin D is a principal factor required for the development and maintenance of bone. In recent years vitamin D has received more attention due to the increased incidence of vitamin D deficiency and rickets in developed countries together with the identification of the involvement of vitamin D in diverse cellular processes, suggesting numerous benefits of vitamin D in health and disease. The possibility of effects of vitamin D beyond bone was first noted with the discovery of the vitamin D receptor (VDR) in tissues and cells that are not involved in maintaining calcium homeostasis including breast, prostate and colon cancer cells and activated T cells. Evidence in the laboratory, including use of animal models, indicates that the hormonally active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃ or calcitriol) can generate a number of extraskeletal effects including inhibition of cancer progression, effects on the cardiovascular system, modulation of innate immunity with subsequent killing of bacteria and inhibition of certain autoimmune diseases. My lab, which has been funded by the NIH for more than 30 years, has investigated the mechanisms by which inadequate vitamin D status contributes to osteoporosis (the principal focus of my research) as well as the possible role of vitamin D in cancer, immunity and autoimmune diseases.

The actions of 1,25(OH)₂D₃ are mediated by the VDR, which together with coregulatory proteins interacts with vitamin D response elements in the DNA of vitamin D target genes, resulting in increased synthesis of proteins involved in maintaining calcium balance. My lab has shown that tissue and gene specific functions of 1,25(OH)₂D₃ are mediated by differential

**Immunocytochemical staining in duodenum for the vitamin D induced calcium binding protein calbindin-D9k (left panel).** There is loss of apical surface staining for calbindin in mice null for the epithelial calcium channel TRPV6 (right panel), suggesting a functional interrelationship between calbindin and TRPV6.
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recruitment of coregulatory proteins to the VDR as well as to epigenetic control (inheritable changes in gene expression that are not mediated at the DNA sequence level). My lab is currently examining molecular level changes in kidney and intestine that are involved in the dysregulation of calcium homeostasis that occurs with aging. The long-term goal is to define molecular pathways of vitamin D action in order to reveal new therapeutic strategies to sustain calcium balance. Identification of the most effective vitamin D analog to prevent or reverse deterioration of calcium homeostasis through transcriptional and epigenomic mechanisms may have long-term implications for how bone diseases, particularly osteopenia and osteoporosis, are treated.

With regard to non-classical actions of 1,25(OH)2D3, numerous studies have shown that 1,25(OH)2D3 can exert inhibitory effects on the growth of a number of malignant cells. My lab has shown that C/EBPα, a transcription factor that has been shown to play a critical role in growth arrest of other cell types, is induced by 1,25(OH)2D3 in MCF-7 human breast cancer cells. C/EBPα was found to induce the transcription of the VDR in MCF-7 cells. Since the levels of VDR correlate with the antiproliferative actions of 1,25(OH)2D3 and since it has been suggested that C/EBPα can be considered a potential tumor suppressor, these findings suggest mechanisms whereby 1,25(OH)2D3 may act to inhibit growth of breast cancer cells. These findings also identify C/EBPα as a 1,25(OH)2D3 target in breast cancer cells and provide evidence for C/EBPα as a candidate whose is associated with an inhibition of IL-17. The mechanism of suppression of IL-17 by 1,25(OH)2D3 involves, at least in part, a competition of VDR with NFAT (a transcription factor that activates IL-17). Since clinical trials are being done treating MS patients with high dose vitamin D, we are currently examining the effect of high dose dietary vitamin D (in combination with interferon beta, an approved treatment for MS) on paralysis, on the progression of EAE and on the production of IL-17 and other cytokines. These studies will increase our understanding of the interaction between the vitamin D endocrine system and the immune system and may suggest new therapeutic targets for treatment of MS and other Th17 dependent inflammatory diseases. 1,25(OH)2D3 not only regulates adaptive but also innate immunity. 1,25(OH)2D3 induces the antimicrobial peptide cathelicidin, with subsequent killing of bacteria.

Recently we found that C/EBPβ is a potent enhancer of cathelicidin antimicrobial peptide gene transcription and that C/EBPβ functionally cooperates with the VDR in the regulation of cathelicidin in lung epithelial cells. Thus, there is increasing evidence that C/EBP isoforms may be key mediators of 1,25(OH)2D3 action in different suggests therapeutic targets. Although, unlike rickets, there is not a causal link between vitamin D deficiency and certain diseases such as cancer and MS, evidence in the laboratory of beneficial effects of 1,25(OH)2D3 beyond bone is convincing. Findings in animal models may suggest pathways in humans that could lead to new therapies.

Sylvia Christakos received her PhD from the State University of New York (SUNY) at Buffalo School of Medicine. She completed her post-doctoral training at the Roswell Park Memorial Institute, Buffalo, SUNY Buffalo School of Medicine Department of Biochemistry, and at the University of California at Riverside Department of Biochemistry. Dr. Christakos has received continuous funding from the NIH for the past 30 years. Her laboratory is one of the leading laboratories involved in research related to vitamin D, its function and mechanism of action.
From the transformation event to metastatic disease, a tumor cell exists in a constant state of damage control. One of the best studied properties of a cancer cell that illustrates this point is the elevated reactive oxygen species (ROS) levels observed in several cancer types. Increased ROS levels are most likely the result of several factors including increased metabolism through aerobic glycolysis (Warburg effect), electron chain dysfunction, cytokine activation and inflammation. In addition, cancer cells are subjected to oxidative stress due to nutrient deprivation and hypoxia due to poor vascularization. Since excessive ROS can induce cell death or growth arrest through activation of DNA damage or other stress checkpoint pathways, these systems are often inactivated by mutation (e.g., p53, BRCA1) in cancer cells. These findings make it clear that handling oxidative stress-induced damage is an important aspect for both the initiation and progression of cancer.

Many current anti-cancer drug strategies attempt to generate sufficient cellular damage to induce programmed cell death (PCD) in the tumor. Studies over the past decade have revealed that the mitochondria are a key regulatory hub for PCD. Mitochondria are dynamic organelles undergoing constant fusion and fission during normal cell division. The equilibrium between fission and fusion is controlled by the activity of conserved molecular machines driven by dynamin-like GTPases. Drp1 forms atypical helical filaments that encircle and constrict mitochondria until scission is achieved (Figure 1, top panel). However, in response to stress, this balance is shifted toward fission to produce highly fragmented mitochondria (middle panel). Extensive mitochondrial fission is a hallmark of the apoptotic response conserved from yeast to mammals and has been proposed to be associated with loss of mitochondrial membrane integrity and release of pro-apoptotic factors (e.g., cytochrome c). Given the importance of mitochondrial fission for initiating the PCD pathway, the molecular trigger responsible for inducing this extensive fragmentation may represent a useful target for therapeutic intervention.

Cyclin C-Cdk8p is a conserved protein kinase that associates with the RNA polymerase II holoenzyme Mediator complex and plays a role in the second job of Cyclin C, life beyond transcription.
both a positive and negative role in transcription. As expected, cyclin C is found in the nucleus in unstressed mouse embryo fibroblasts (MEF; Figure 1). However, exposing these cells to low level oxidative stress in the form of hydrogen peroxide (H2O2) induces a relocalization of a portion of cyclin C out of the nucleus into the cytoplasm. In the cytoplasm, cyclin C associates with the mitochondria (arrows in insert) and induces fragmentation of this organelle. This interaction is important as either yeast or mouse cells deleted for cyclin C fail to undergo fission and are protected from oxidative stress-induced cell death.

Based on our results from yeast and mammalian cell systems, we propose a two-step model to describe cyclin C-dependent control of PCD. Under normal conditions, cyclin C is part of a highly conserved complex with Cdk8 to control gene expression (Figure 2, top panel). The mitochondria are undergoing fission and fusion at a steady rate that produces long, branched, structures. Step 1 predicts that cellular damage, such as ROS or anti-cancer drugs, activates a signaling pathway that triggers the disassociation of cyclin C from Cdk8 and the rest of the transcription machinery (Figure 1, middle panel). Once free of transcription machinery, cyclin C next translocates to the cytoplasm where it helps recruit the fission protein Drp1 to the mitochondria. The efficient association of Drp1 induces extensive mitochondrial fragmentation. However, hyperfission alone is not sufficient to induce cell death. Rather, an addition signal (step 2) is necessary to induce loss of mitochondrial integrity, allowing the release of sequestered proteins necessary for completing PCD execution. Therefore, the activity of cyclin C sensitizes the cell to go down the cell death pathway if the damage is too great to repair.

Most chemotherapeutic regimens attempt to elicit PCD by first inducing cellular damage. However, this approach requires a balancing act between administering sufficient drug concentrations able to eliminate the tumor while still being tolerated by the patient. An alternative approach is to identify drug targets in the PCD control pathway whose activity can be manipulated. Several studies have found that MTX, an intervention protects cells from oxidative stress-induced cell death. However, these approaches inactivating the core fission machinery through mutation or pharmacological intervention protects cells from PCD. However, these approaches cause aberrant mitochondrial morphology resulting in organelle dysfunction including reduced mitochondrial DNA integrity. Therefore, a more effective approach to exploit the requirement of extensive mitochondrial fragmentation for normal PCD is to identify a regulator required only for stress-induced hyper-fission but not normal mitochondrial division. Therefore, understanding how cyclin C regulates mitochondrial dynamics and promotes PCD may provide new targets for therapeutic intervention that can enhance the effectiveness of anti-cancer drugs or protect normal cells from the toxic actions of these treatments.

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here are many barriers facing college students with psychiatric conditions, ranging from stigma to lack of specialized services to the effects of the disorder itself. Research is being conducted to investigate the barriers associated with this sub-group of college students to enhance support services in order to minimize the impact of these diagnoses on degree completion.

Each year about one in five Americans experiences a diagnosable psychiatric disability, including major depressive disorders, bipolar disorder, schizophrenia and a range of anxiety disorders. There is also increasing evidence that the number of college students diagnosed with psychiatric disabilities is quickly rising, yet there is little empirical research on how to help these students succeed. In a 2010 survey of more than 190 U.S. colleges and universities, nearly 35,500 students reported being diagnosed with a psychiatric impairment within the previous 12 months.

Psychiatric conditions are greatly stigmatized in our society. On college campuses, this stigma influences the attitudes of administrators, staff, faculty and students, causing many students with psychiatric conditions to hide their disorders on campus, frequently dropping out of school instead of receiving needed services. In response to incidences of gun violence on campuses over the last few years, many colleges and universities have implemented withdrawal policies for students with acute symptoms. Presumably, the intention of these policies is to make the campuses safer. However, such policies actually reduce the likelihood that students will reach out for help either for themselves or for their friends, and can end up heightening fear among students of those who are struggling with symptoms.

Section 504 of the Rehabilitation Act of 1973 is a national law that protects qualified individuals from discrimination based on their disability. This act ensures that individuals with disabilities have access to higher education that is equal to their peers without impairments. Disability services were initially focused on those with physical disabilities and, since the 1990s, have included those with learning disabilities. Campuses now have more students with more complex psychiatric diagnoses, but many colleges and universities do not have sufficient staff with highly specialized knowledge and training to adequately provide services to them.

Students with psychiatric diagnoses are not a uniform group. Some are “typical” college-age students, in their late teens and early twenties, who are grappling with the onset of their illness and the necessary adjustments. Other typical college-age students were diagnosed at an earlier age. Still others may be non-traditional students, who are older and are struggling to adapt to the classroom they left decades ago. The same psychiatric diagnosis affects people in many different ways, creating a complicated profile for students, faculty and support staff to understand.

In order to address some of these issues, I am conducting four studies whose overarching question is whether specialized interventions will improve the postsecondary success and career achievement of students with psychiatric conditions. Over the next five years the National Institute on Disability and Rehabilitation Research (NIDRR) will provide us with $3.5 million to study and develop these interventions.

Dr. Kenneth Gill and I are currently conducting a study, Effectiveness of Educational Supports on Retention of Postsecondary Students with Psychiatric Disabilities. It is the first, multi-site, randomized control trial of what is known as “Supported Education.” It is funded in partnership with Temple University’s Rehabilitation Research and Training Center (RRTC) on Community Inclusion. This study compares a specialized educational support intervention for college students (i.e., Supported Education) with an informational program that resembles typical services provided by Disability Services. It addresses the question of whether enhanced services and support help students stay in school, complete courses, earn degrees and move on to successful employment.

While analyzing data from the Effectiveness of Educational Supports study, we became aware of widespread cognitive deficits in the students with diagnosed psychiatric disorders. A high percentage of participants reported struggling most with concentration (85%), time management (75%), prioritizing tasks (75%) and organization (70%), all executive functioning skills. Formerly, it was assumed that helping people manage their psychiatric symptoms, and helping them to bridge the academic and mental health worlds, were sufficient services to provide students affected by psychiatric conditions. However, our study results suggest that these students need targeted skill development to improve their academic performance. Our current project, Developing Executive Functioning through Cognitive Remediation for College Students with Psychiatric Disabilities, evaluates the effectiveness of an intervention that teaches the skills of planning, organization, and time management.
Students’ needs for support services vary based on other factors as well. We have also partnered with the Transitions RRTC at the University of Massachusetts Medical School to evaluate the differences between the educational support needs of young adults compared to older adults with psychiatric conditions. Many young adults have had the support of specialized services in high school, while others experienced the onset of their disorders while in college. Some of the somewhat older adults, returning to school, had their college educations disrupted by frequent or long hospitalizations for their psychiatric disorders. Each student-profile shows different needs, requiring different services and support, and is expected to have different outcomes.

Additionally, we are just beginning a five-year project aimed at creating a comprehensive career development service approach for those in their late teens and early adulthood who have psychiatric conditions. In practice, educational and employment goals are pursued in tandem, separately, or sequentially. Our approach, based on all the available evidence, will provide a model for addressing the career development concerns of these young people in a comprehensive fashion over time.

**Cohesin: Chromosome Glue With Roles in Transcription, Too**

by Marc R. Garfenberg

The mechanical process of chromosome segregation has intrigued biologists for more than a century, long before it was known that chromosomes were the repositories of genetic information. In the last 50 years, significant progress has been made to understand the principle drivers of chromosome segregation: the motors and microtubules of the spindle and the signaling pathways that direct their function. One particular feature of the process, however, has remained enigmatic until recently. Newly replicated chromosomes, termed sister chromatids, hold onto each other right up to the moment chromosomes segregate, at which point all sister chromatid pairs separate synchronously. It was not known how sister chromatids hold on to one another and what triggers their ultimate separation.

Answers to these questions began to emerge 15 years ago with the discovery of cohesin, the DNA-bound protein complex that forms the molecular glue between sister chromatids. Cohesin complexes become active as the DNA replication machinery traverses chromosomes and they remain active until the onset of chromosome segregation when the complexes are removed or destroyed. The shape of cohesin is striking. The four subunits assemble into a large donut-shaped structure with an opening at the center large enough to hold two DNA duplexes. Current evidence suggests that cohesin holds pairs of sister chromatids together by creating an embrace around the DNAs, at least at some locations.

Cohesion of sister chromatids contributes to chromosome segregation fidelity by assuring that each sister chromatid attaches properly to spindle microtubules. Certain defects in the complex way the complex binds DNA and brings distant DNA sites together. My lab has long been interested in understanding how the structural features of chromosomes relate to gene expression. Our approaches take advantage of the powerful experimental methods available for studies in the genetically tractable baker’s yeast *Saccharomyces cerevisiae*. Conservation of the underlying principles of chromosome biology in all eukaryotes allows us to use baker’s yeast to study matters relevant to human health and disease.

Cohesin binds densely around centromeres, where microtubules meet chromosomes, to assert its role in chromosome spindle attachment. The complex also binds other discrete locations along yeast chromosomes but the molecular basis for this site selection is not understood. Significantly, historically, it was assumed that students with psychiatric conditions drop out of college primarily because of their symptoms, yet this is far from clear. Other barriers and factors are involved that are secondary to symptoms, and some are even unrelated to their disorders. All academic environments need to openly discuss mental health issues in higher education, seek information to help their faculty and administration understand that those with psychiatric conditions are not dangerous, better understand the struggles of their students, and identify services and supports that assist them in maintaining matriculation. Through our research we hope to influence the types and quality of services provided to the growing number of students with psychiatric conditions.

Michelle G. Mullen-Gonzalez is currently conducting four research projects exploring the services and practices of Supported Education (SEd). She is the statewide trainer for the SEd programs in New Jersey designed specifically to help students with psychiatric disabilities maintain college matriculation and has provided training programs nationally and internationally related to college students with psychiatric conditions.
changes in the transcriptional program of the cell can alter the positions of these non-centromeric binding sites. My lab is currently interested in how cohesin binding sites are specified, how the donut-shaped complex binds these sites and how the complex moves between different locations.

Our work is progressing on three fronts
1) Chromosomes contain large domains of heterochromatin, a condensed structure that hinders expression of genes nestled within its borders. In baker's yeast, heterochromatin domains near the chromosome ends repress genes that determine mating behavior. We found that sister chromatids are held together by cohesin at these locations. Our data indicated that the donut-shaped complex embraces heterochromatic DNA but not in a way that embraces both sister chromatids simultaneously. We concluded that cohesin possesses more than one DNA binding mode and that chromosomal context determines how the complex binds DNA. Further work in my lab showed that cohesin is targeted to heterochromatin by Sir2, an enzyme that catalyzes heterochromatin assembly by modifying fundamental chromosome proteins known as histones. Sir2 is highly conserved. In a variety of organisms, Sir2 family members participate in numerous pathways that prevent disease states and extend lifespan. In yeast we found that recruitment of cohesin by Sir2 accelerates transcriptional repression within heterochromatin. We do not yet know the mechanistic basis for this behavior. One possibility is that cohesin concentrates both chromatids together within a microenvironment of the nucleus that favors heterochromatin assembly.

2) The vast majority of cohesin binding sites in the yeast genome are situated at intriguing positions relative to genes, often at the ends of genes transcribed by RNA polymerase II. My lab is investigating the basis for these binding characteristics using an archetypal yeast gene known as URA3. Expression of the gene elevates when cells are starved for uracil. In contrast to the situation at heterochromatin, a single transcriptional activator binds the URA3 gene and recruits cohesin to DNA. We speculate that other transcriptional activators do the same, probably through the action of a shared mediator as suggested from work in human cells. Remarkably, cohesion is lost when the URA3 gene is activated. Our results are consistent with RNA polymerase II pushing cohesin complexes that embrace DNA off the end of the gene. This interpretation is reinforced by the use of URA3 DNA mini-circles, which prevent transcription-driven loss of functional cohesin because DNA circles lack DNA ends.

3) tRNAs of baker's yeast are encoded by more than 250 RNA polymerase III-transcribed genes. Despite their wide distribution across every chromosome, the genes frequently coalesce with one another in subcompartments of the nucleus. My lab and others uncovered an additional role for tRNA genes in chromosome architecture: the genes act as chromosomal entry sites for cohesin complexes that, once bound to DNA, migrate along DNA to their final destinations. During these studies we made an unexpected observation: tRNA gene expression fluctuates during cell cycle progression with transcription peaking in mitosis. Moreover, we found that when transcription is highest the tRNA genes move to nuclear pore complexes at the nuclear membrane (nuclear membranes do not disassemble at mitosis in yeast). The spatial reorganization requires cohesin, even for RNA genes that don’t coalesce with others. Previous work showed that some genes transcribed heavily by RNA polymerase II also associate with nuclear pore complexes. In both cases, docking at these sites may facilitate rapid export of RNAs from the nucleus to the cytoplasm where they are used in protein synthesis.

The embrace of DNA by cohesin provides an elegant solution to how the protein complex glues sister chromatids together while retaining the ability to slide between different sites on the same DNA. It will be exciting to learn how these characteristics are used by cohesin in the increasing number of roles the complex plays in transcription and other chromosomal transactions.

In addition to being a professor in the newly formed Department of Biochemistry and Molecular Biology, Marc Gartenberg is the director of the Graduate Program in Cellular and Molecular Pharmacology. He earned a PhD from Yale University and carried out post-doctoral research at Harvard University. He joined the faculty of Robert Wood Johnson Medical School in 1993. His lab uses molecular genetic and cell biological methods to study chromosome structure and function in a model eukaryotic organism, the baker’s yeast Saccharomyces cerevisiae.
My journey in lung cancer research began during my postdoctoral fellowship at the NIH, under the mentorship of Curtis C. Harris, MD, when the popularity of the cancer stem cell (CSC) hypothesis in solid tumors was making its debut. The CSC hypothesis was initially derived from observations that only a certain rare population of cells within a tumor was capable of initiating new tumors. CSCs are more resistant to traditional therapies compared to the bulk tumor cells and it is necessary to eliminate them to improve survival in cancer patients. I was intrigued by recent data in the literature from the K-ras-induced lung cancer mouse model in which tumors were arising at a location within the lung, at the junction between the alveoli and bronchioles, believed to harbor lung epithelial stem cells. Although the resulting tumors emerge from hyperplastic sites comprised almost exclusively of expanded stem cells, the tumors themselves comprise heterogeneous malignant cells, including stem and differentiated cells. These observations led me to wonder how CSCs give rise to the bulk of the differentiated tumor, while also maintaining their own self-renewing pool. I quickly learned this was an unanswered question that had puzzled scientists across the entire cancer field.

My experience in normal stem cell biology during my PhD training reminded me that during embryonic development of lower organisms, stem cells undergo asymmetric cell division to produce one daughter cell that remains a stem cell, while the other daughter differentiates into a specialized cell type. When asymmetric cell division is disrupted in these lower organisms, hyperplastic lesions and tumors form due to excessive symmetric divisions of the stem cells. From this, I hypothesized that cancer results from asymmetric divisions gone awry — but some remnants of asymmetric cell division control are still retained. If it was true that CSCs retain the ability to choose the fate of their daughters, then that decision process could be targeted during therapy, and the CSC pool could be depleted. I did not know at the time that this work would lead mental biology to help solve this puzzle. The Notch pathway is a key regulator of asymmetric cell division during embryonic development of lower organisms. We found that knock-down of Notch activity results in loss of asymmetric cell division in lung cancer, providing us with a tool to interrogate the molecular mechanisms driving self-renewal. We were intrigued by other recent literature that the Notch pathway is upregulated in tumor cells when patients are treated with chemotherapeutic drugs, such as cisplatin. Through bio-informatics analyses and basic biochemistry research, we are also identifying novel Notch pathway target genes responsible for mediating the effects of Notch signaling on lung cancer cell self-renewal and survival. In collaboration with John Langenfeld, MD, we have collected primary lung tumor tissues and developed a series of patient-derived lung tumor xenograft lines. We are performing pre-clinical trials testing the efficacy of novel Notch inhibitors alone, and in combination with standard therapies. The knowledge gained from these studies promises to improve lung cancer therapies, through targeting CSC self-renewal and therapy resistance pathways, and increase the survival rates for lung cancer patients.

A putative lung cancer stem cell labeled with green fluorescent protein undergoes self-renewal when it asymmetrically divides. The cancer stem cell stands out from surrounding differentiated lung cancer cells expressing red fluorescent protein localized to their cell membranes.

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There are many definitions of research and many different types of research, but, simply stated:

Research is a “studious inquiry or examination, especially: an investigation or experimentation aimed at the discovery and interpretation of facts, a revision of accepted theories or laws in the light of new facts, or a practical application of such new or revised theories or laws.”

(Miriam Webster Dictionary)

The word research comes from the “Middle French recercher, to go about seeking.”

We want to thank the more than 200 researchers and their teams who took time from their “seeking” to communicate to the UMDNJ Research audience what goes on in their labs and what they aim to achieve through their “amazing science.” Since this publication was launched in 2000, the response to the bylined articles and the photos of the researchers and their teams has been phenomenal. Opening the doors to their labs — figuratively speaking — has provided our readers with a rare glimpse into the process of discovery behind the “breakthrough” science that impacts every aspect of our lives every day.