Joint Molecular Biosciences Graduate Student Association

9th Annual Graduate Student Research Symposium

Friday March 27, 2015
9:30AM - 4:30PM
Life Sciences Building Atrium
Message From The Organizers

Welcome to the 9th Annual Graduate Student Symposium hosted by the Joint Molecular Biosciences Graduate Student Association (JMBGSA) of Rutgers University. We are delighted to have you join us today to support the outstanding work that graduate students here at Molecular Biosciences Graduate Programs have been producing throughout their different stages of graduate learning.

As a student organization, the goal of JMBGSA is to facilitate the professional development of graduate students, and provide them with opportunities for social interaction with their peers. By allowing them to present their work to a critical audience, graduate students are able to hone their presentation skills, and receive input on their work from faculty from various departments. With these goals in mind, we organize the annual symposium, and each year we look to the university community to make it a success.

Through this symposium, we hope to not only showcase the graduate student research, but also to provide a platform for professional interaction between students, faculty and administration. We are proud to be able to provide the avenue for students from different labs to showcase their scientific endeavors across different fields of bioscience research. Through both the oral and poster presentations, we sought to introduce some of the interesting basic and translational science conducted at Rutgers.

We have received tremendous help and support from our peers and faculty alike in putting this symposium together. We thank our advisor Dr. Janet Alder for her help and encouragement during the planning process. We also thank the graduate student offices for their tremendous support of our program. We gratefully acknowledge the faculty members who have taken time out from their busy schedules to participate as judges and give students feedback on their work. Since this event would not be possible without the participation of our fellow-graduate students, we applaud their efforts and thank them for their poster presentations and talks. We’d like to offer a very special thank you to all our generous sponsors as well for supporting graduate student research at Rutgers.

Thank you for joining us today. We sincerely hope that you will enjoy this year’s Annual Graduate Student Symposium and we hope to see you next year.

Sincerely,

JMBGSA Executive Board 2014-2015

[Images of JMBGSA Executive Board members]
Message From The Faculty Advisor

On behalf of the faculty at Rutgers, I would like to welcome you to the 9th Annual Joint Molecular Biosciences Graduate Student Association Symposium. Rutgers has many talented graduate students and we embrace this opportunity to showcase the cutting-edge scientific research that our students are performing. The annual symposium also serves as a learning opportunity for our students practice their presentation skills to their peers and the faculty. By giving awards for best oral and poster presentations at the symposium, we are able to highlight some graduate students that excel in research and communication skills.

The JMBGSA is an important organization that serves several purposes on campus. It provides social programming for the graduate students to create a sense of community that lasts beyond the first year of graduate school. These interactions with your peers are critical to success in graduate school and future collaborations among colleagues. The JMBGSA also invites excellent speakers and hosts events to enrich the scientific atmosphere on campus. This year we are honored to have a distinguished alumnus of our program, Ronny Drapkin, MD/PhD as our keynote speaker at the symposium. The success of our alumni highlights the quality education they received here at Rutgers.

We are very grateful for the leadership of the JMBGSA Executive Board. These graduate student volunteers including Na Cai (President), Malan Silva (Vice President), Almin Lalani (Secretary), Megha Shettigar (Treasurer) and Jeremy Tang (Webmaster) put in countless hours to provide innovative and relevant programming for their peers. The organizational and leadership skills they are acquiring in their volunteer positions will help them in their future careers and we encourage all graduate students to get involved in the JMBGSA or other students organizations.

Thank you to the sponsors and organizers of this year’s symposium and best of luck to the presenters.

Janet Alder, PhD
Faculty advisor to JMBGSA
Director of Academic and Student Affairs,
Rutgers Graduate School of Biomedical Sciences
Associate Professor, Neuroscience and Cell Biology,
Rutgers Robert Wood Johnson Medical School
PROGRAM

09:30 – 09:55 am  | Registration and Breakfast
09:55 – 10:00 am  | Introductory Remarks
10:00 --11 :00 am | Oral Presentations I

10:00 – 10:15 am  | Phosphorylation Requirement of Murine Leukemia Virus p12
                  | Jon Brzezinski

10:15 – 10:30 am  | Analysis of Human Aurora Kinase C Variants in Oocyte Meiosis
                  | Jessica Fellmeth

10:30 – 10:45 am  | Regulation of Macrophage Activity by Histone Deacetylases during Nitrogen Mustard-Induced Lung Injury
                  | Alessandro Venosa

10:45 – 11:00 am  | Monitoring Collagen Fibrillogenesis With Circular Dichroism Spectroscopy
                  | Kathryn Drzewiecki

11:00 – 12:00 pm  | Poster Presentations I
12:00 – 01:00 pm  | Lunch
01:00 – 02:00 pm  | Keynote Speech

2:00 – 3:00 pm    | Poster Presentation II
3:00 - 4:00 am    | Oral Presentations II

3:00 – 3:15 pm    | Functional Dissection of VapBC Toxin-Antitoxin Systems in Mycobacterium tuberculosis
                  | Melvili Cintron

3:15 – 3:30 pm    | Synaptonemal Complex Assembly is Regulated by Multiple Cohesion Complexes in Drosophila Meiosis
                  | Mercedes Gyuricza

3:30 – 3:45 pm    | Zbtb16 (PLZF) is Stably Suppressed and Not Inducible in Non-innate T cells via T cell Receptor-mediated Signaling
                  | Sai Zhang

3:45 – 4:00 pm    | Development of Improved Metric for Traumatic Brain Injury Monitoring
                  | Maria Qadri

4:00 – 4:30 pm    | Award Presentation

Keynote Speech

Dr. Ronny Drapkin, MD, PhD

Non-ovarian origins of ovarian cancer: How knowledge of pathogenesis is driving novel therapeutic opportunities

Two travel awards for top poster and oral presentations
Four giftcards for distinctive poster and oral presentations
Two travel awards for distinctive presentations in Reproductive Genetics
Phosphorylation Requirement of Murine Leukemia Virus p12

Brzezinski, J; Felkner, R; Roth, M

The p12 protein of murine leukemia virus (MLV) Gag is associated with the preintegration complex (PIC), and mutants of p12 (PM14) exhibit defects in nuclear entry/retention. We have shown that p12 is responsible for tethering the PIC to the host chromatin, and here we show that a phosphorylated peptide motif derived from human papillomavirus 8 (HPV 8), the E2 hinge region (240-255), can functionally replace the main phosphorylated motif of MLV p12 and can rescue the viral titer of the lethal p12-PM14 mutant. This HPV 8 motif is responsible for modulating DNA tethering in the E2 protein. Complementation with the HPV 8 E2 hinge motif generated multiple viral revertants in live viral passage assays, including one deleting the HPV 8 E2 phosphorylation motif. The phosphorylation of this and two other revertants are investigated by mass spectrometry and Western blotting. In addition, the DNA binding of these and individual site mutations of the p12 phosphorylation motif are shown via p12-GFP confocal imaging. We found that the native phosphorylation of p12 is required for its DNA binding function and viral fitness, and that deletion of this motif gives rise to viral revertants that rescue fitness and DNA tethering.

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Analysis of human Aurora kinase C variants in oocyte meiosis

Fellmeth, Jessica, Treff, Nathan R., Schindler, Karen

The Aurora protein kinases are well-established regulators of chromosome segregation. Our studies in mouse oocytes indicate that AURKC is required to regulate chromosome segregation during meiosis I (MI); however, little is known about the functional significance of AURKC in human oocytes. In this study, we use Aurkc-/- oocytes to address the function of AURKC splice variants and sterility-associated variants.

To evaluate the functional differences between the splice variants, we created GFP-tagged constructs to express in oocytes. By quantifying metaphase chromosome alignment, cell cycle progression, phosphorylation of INCENP, and microtubule attachments to kinetochores, we found that AURKC_v1 is the most capable of the 3 variants at supporting MI chromosome segregation although AURKC_v2 is the most stable splice variant.

Three mutations in the coding region of Aurk (L49W, C229Y, and Y248X) are correlated with male infertility. These men have 100% tetraploid sperm with multiple flagella. We found that neither the L49W or Y248X mutants localized to the chromosomes when expressed in Aurk-/- oocytes; however, C229Y localized to centromeres in most oocytes and phosphorylated INCENP indicating that C229Y retains partial kinase activity.

AURKC plays a critical role in maintaining ploidy in oocytes. Here we show 2 splice variants that support this role in separate ways: v1 through error correction and v2 through protein stability. We also show that when its function is altered by mutation in the kinase domain, that fidelity of chromosome segregation is impaired.

This study was supported by an ASRM Research grant and a Busch Biomedical Foundation grant to K.S.

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Regulation of Macrophage Activity by Histone Deacetylases during Nitrogen Mustard-Induced Lung Injury

Alessandro Venosa, James Gow, Ian Berman, Rama Malaviya, Hakan Yaren, Halil Yaman, Andrew Gow, Jeffrey Laskin, Debra Laskin

NM is a bifunctional alkylating agent which causes acute lung injury and fibrosis. Proinflammatory/M1 and antiinflammatory/M2 macrophages have been implicated in NM-induced lung toxicity. M1 and M2 macrophage activation is controlled at the chromatin level by histone deacetylases (HDACs) and histone acetyltransferases. Herein, we analyzed the role of chromatin modification in NM-induced lung macrophage activation and toxicity. Rats were exposed to PBS or NM (0.125 mg/kg) followed 30 min later by valproic acid (VA, 300 mg/kg/day), a class I HDAC inhibitor. VA suppressed NM-induced upregulation of HDAC2 in the lung, but increased histone H3-lysine9-acetylase (H3K9Ac). This was associated with a decrease in bronchoalveolar lavage (BAL) cell content, a marker of inflammation and histologic evidence of tissue injury. VA also caused a reduction in iNOS+ proinflammatory/M1 macrophages in the lung after NM and reduced M1 gene (iNOS, IL-12, COX2, MMP-9) expression. Conversely, CD68+ and CD163+ antiinflammatory/M2 macrophages and M2 genes (IL-10, ApoE) increased. Flow cytometric analysis of isolated lung macrophages revealed two subpopulations in the lung after NM: mature antiinflammatory (CD11b+CD43-) and immature proinflammatory (CD11b+CD43+) cells. Whereas at 3 d post NM, VA reduced numbers of mature antiinflammatory macrophages, at 7 d these cells were increased; at this time there was also a reduction in immature proinflammatory macrophages and in resident alveolar macrophages. These studies show that HDACs contribute to regulating macrophage phenotype and activation during the pathogenesis of NM-induced lung injury; this may contribute to the toxicity of this vesicant.

Supported by NIH AR055073, HL096426, ES004738, CA132624 and ES005022.

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Monitoring Collagen Fibrillogenesis With Circular Dichroism Spectroscopy

Kathryn E. Drzewiecki, Vikas Nanda, David I. Shreiber

Circular dichroism (CD) spectroscopy is a method utilized to estimate the secondary structure of proteins. Briefly, left-handed and right-handed circularly polarized light is absorbed differently by asymmetric molecules, which results in characteristic CD spectra for specific protein structures. Type-I collagen, a protein found ubiquitously in the body, is known to be triple-helical in its monomeric and fibrillar state, and has a characteristic CD spectra with a positive peak mean residue ellipticity (MRE) at 222 nm. We have identified a unique spectroscopic signal that may be an indicator of collagen fibrillogenesis. The triple-helical content of collagen in acidic or physiological buffer was monitored as the temperature was increased from 4 °C to 60 °C. Collagen in acidic buffer maintained triple-helix signal until denaturation (MRE ~ 0) at temperatures above 40 °C. In contrast, collagen in physiological buffer exhibited a negative MRE (or loss of triple-helix) signal at temperatures that promote collagen fibrillogenesis (above 30 °C), and denatured at higher temperatures (50 °C). Further, temperature-dependent shear rheology studies demonstrated gel formation as an increase in storage modulus at the same temperatures as the negative MRE signal was seen in CD experiments. Other sources, species, and types of collagen were tested to examine this phenomenon, and overall, any condition that supported collagen fibrillogenesis exhibited this MRE transition. This unique spectroscopic signal that we discovered can be utilized to assay the kinetics of collagen fibrillogenesis, fibril stability, and act as a molecular marker that may provide future insight into the method of collagen fibrillogenesis.

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Functional Dissection of VapBC Toxin-Antitoxin Systems in 
Mycobacterium tuberculosis

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One third of the world’s population is infected with Mycobacterium 
tuberculosis (Mtb), the pathogen responsible for tuberculosis (TB). Its 
ability to persist within its host for extended periods of time as a latent 
infected makes Mtb one of the most successful pathogens in history. 
Identifying the molecular mechanisms that underlie Mtb latency is 
essential for developing new therapeutics. The activity of the toxin 
component of toxin-antitoxin (TA) systems has been implicated in the 
slow growth, stress response and non-replicating persistent state that 
are hallmarks of latent TB. Mtb harbors 48 members of the VapBC 
family of TA systems. The VapC toxin component contains a conserved 
catalytic PIN domain that is associated with nuclease activity. We are 
studying a representative of the VapC toxin family, VapC-mt19, to better 
understand the general mechanism of action of such toxins in Mtb. We 
first determined that VapC-mt19 is an isoacceptor-specific tRNase. We 
also demonstrated that the substrate specificity of VapC-mt19 requires 
both sequence and structural determinants. Finally, we hypothesize that 
the presence of these tRNases may underlie reprogramming of the Mtb 
proteome during latent TB.

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Abstract

Synaptonemal complex assembly is regulated by multiple cohesion complexes in Drosophila meiosis

Gyuricza, MR, Landy, KB, McKim, KS

The cohesin complex, a ring-like structure comprised of four subunits, SMC1, SMC3, Rad21, and Stromalin, holds sister chromatids together. During meiosis homologous pairs of chromosomes are held together the synaptonemal complex (SC). It has been shown previously that formation of the SC is dependent upon SMC1 and SMC3. SC is also dependent upon C(2)M and ORD both proteins also found at the chromosome axis. This is of interest because C(2)M is a kleisin. Kleisins are a group of proteins known to associate with SMCs and aid in closing the rings. Therefore, because of the patchy SC phenotype in C(2)M mutants it is proposed that C(2)M is functioning as the kleisin in some but not all of the cohesin complexes in Drosophila meiosis. stromalin RNAi results in an intermediate, patchy, SC phenotype. We are looking further into the intermediate phenotype by making a null mutation in stromalin and characterizing the phenotype. ORD however, has a very different phenotype, threads of SC. Indicating that it may be involved with a different population of cohesins. The differing phenotypes of the four ring subunits suggest that there may be multiple cohesin complexes in Drosophila meiosis. We are currently characterizing the components of each of the cohesin complexes. We also have evidence that indicates that some of these complexes containing C(2)M are dynamic and are only required for homolog interactions whereas others are required for sister interactions. We are looking at other components to determine if they are static or dynamic.

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Zbtb16 (PLZF) is stably suppressed and not inducible in non-innate T cells via T cell receptor-mediated signaling

Zhang, S, Laouar, A, Denzin, LK, Sant'Angelo, DB

Invariant natural killer T (NKT) cells are distinct from conventional T cells in many ways, including their rapid and potent response to antigen. It is now understood that the transcription factor PLZF (promyelocytic leukemia zinc finger; zbtb16) is essential for nearly all of the unique characteristics of NKT cells. In the immune system, zbtb16 expression is only found in innate cells. Conventional T cells that ectopically express PLZF spontaneously acquire an activated, effector phenotype. Activation induced expression of lineage defining transcription factors such as T-bet, FoxP3, RORγt, GATA3 and others is essential for naïve T cell differentiation into effector T cells. In this study, we used sensitive genetic-based approaches to assess the induction of PLZF expression in non-innate T cells by T cell receptor (TCR)-mediated activation. Surprisingly, we found that PLZF was stably repressed in non-innate T cells and that TCR-mediated signaling was not sufficient to induce PLZF in conventional T cells. The inactivated state of PLZF was stably maintained in mature T cells, even under inflammatory conditions imposed by bacterial infection. Collectively, our data show that PLZF expression is highly specific to innate T cells and cannot be induced in conventional T cells via TCR-mediated activation or inflammatory challenge.

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Abstract

**Development of Improved Metric for Traumatic Brain Injury Monitoring**

Qadri, M. J., Kim, N. H., Kosinski, C., Danish, S., Wininger, M., Craelius, W.

In monitoring severe traumatic brain injury patients in the surgical intensive care unit, the target is to ensure normal intracranial pressures (ICP) as blood pressure varies in order to maintain adequate tissue oxygenation – a task performed by the cerebrovascular control system autoregulation (AR). Detecting the state of AR in a patient is impossible without artificially altering patient physiology and risking patient safety. Instead, patients are monitored continuously to detect lapses in AR, and they are only treated after a lapse occurs. Current monitors use simple thresholds to alert caregivers of elevated ICP. Our goal is to develop metrics that better capture current ICP behavior and can predict future ICP trends in order to improve patient outcomes. Previous studies show that phase domain analysis of repetitive waves can yield much greater discrimination sensitivity than analysis in the time domain. To utilize this insight, we have developed a single metric of ICP cycle regularity using phase plane analysis called the phase area ratio (PAR). In addition to developing meaningful artificial signals to test our metric, we have also constructed a device to collect and record physiological data from patient monitors for analysis. We will show the development and testing of PAR using both measured and artificial ICP data as well as preliminary correlation to incidents of ICP hypertension.

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Reversible protein phosphorylation allows cells to direct progression through cell cycle. The significance of protein kinases in cell cycle regulation and progression has been well established. In Drosophila female germ line Aurora B kinase, a member of the chromosomal passenger complex (CPC), is required for meiotic spindle assembly and chromosome segregation. We have also seen that if an Aurora B inhibitor is added after spindle assembly is complete, the spindle completely disintegrates and kinetochore proteins fail to localize. This indicates that continual phosphorylation activity is required due to either the presence of multiple phosphatases or degradation. Hence the protein phosphatases may play equally important reciprocal roles in cell cycle regulation and the processes involved. We have looked at the role of the serine/threonine protein phosphatase 1 (PPI) in female meiotic chromosome segregation and spindle assembly. In PPI depleted oocytes we observe a gross disorganization of spindle microtubules and the length and shape of these spindles resemble prometaphase spindles suggesting a defect in progression to metaphase. In addition the karyosome, a structure into which all the chromosomes are compacted, is broken into several pieces with a loss of sister-centromere cohesion. This is surprising since PP2A is usually required for maintaining cohesion in mitotic cells. Kinetochore proteins like Spc105R however are unaffected by loss of PPI. We have also found that some but not all of these phenotypes are due to phosphorylation by Aurora B. The karyosome defect and loss of cohesion in oocytes lacking PPI is rescued by Aurora B inhibition. In addition the loss of kinetochore protein Spc105R at centromeres upon inhibition of Aurora B is also rescued when PPI is absent. These results suggest that PPI may antagonize Aurora B for maintaining cohesion, karyosome integrity and kinetochore protein localization. However, the complete loss of the meiotic spindle caused by an Aurora B inhibitor is not restored by loss of PPI. Thus, other phosphatases may negatively regulate spindle assembly and/or Aurora B-dependent phosphorylation is required to maintain constant incorporation of spindle associated proteins throughout meiosis.

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 Regulation of channel-kinase TRPM7 by the small signaling protein 14-3-3θ

Cai, N, Overton, J, Runnels, LW

Transient receptor potential melastatin 7 (TRPM7) is a bifunctional protein composing of a TRP ion channel linked to a functional α-kinase domain. Since its identification over a decade ago, the reason why nature endowed the TRPM7 ion channel with its own kinase domain has remained enigmatic. The first hint as to why these two domains are interconnected may have been uncovered by our recent identification of 14-3-3θ as a novel TRPM7 binding protein. 14-3-3θ belongs to a family of conserved regulatory molecules expressed in all eukaryotic cells that regulate signal transduction by binding to phosphorserine/threonine containing proteins. We have identified the binding site for 14-3-3 on TRPM7 and have discovered that the interaction between the two proteins requires phosphorylation of the channel by TRPM7’s own kinase. Mutations that disable 14-3-3θ binding to the channel decrease TRPM7 protein levels, whereas overexpression of 14-3-3 increases protein expression of the channel. We therefore hypothesize that 14-3-3θ is a positive regulator of TRPM7 protein via autophosphorylation-dependent binding to TRPM7. TRPM7 overexpression and over-activity is linked to pathological conditions such as cardiac fibrosis and ischemic neuronal cell death, this study will advance our understanding of TRPM7 function and regulation and may uncover novel strategies for the treatment of these devastating diseases.

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Extracellular matrix genes regulate ciliary integrity and ciliary receptor localization

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic disease in human (frequency 1/400-1/1000) and caused by mutations in the polycystin-encoding genes PKD1 and PKD2. In humans and C. elegans, the polycystins localize to and function in sensory cilia. In the nematode, the polycystins LOV-1 and PKD-2 localize to sensory cilia located at the ends of dendrites in male-specific neurons and are necessary for male-specific mating behaviors. To identify regulators of polycystin ciliary localization, we performed a genetic screen for mutations that disrupt PKD-2::GFP subcellular distribution. Here, I show that extracellular matrix encoding genes mec-1, mec-5, and mec-9 play multifunctional roles required for the function of diverse sensory neuronal types. These ECM genes were originally identified based on their role in mechanosensation mediated by the touch receptor neurons (Du 1996; Emtage 2004). In the male, I found that mec-1, mec-5, and mec-9 act non-redundantly and are required for polycystin ciliary localization and polycystin-mediated male mating behavior. In male and hermaphrodite shared ciliated sensory neurons, I show that these ECM genes also play important roles in regulating ciliary integrity. These results expand the scope of activity of these ECM components and lend insight on how ECM proteins contribute to ciliary localization of sensory receptors like PKD-2 and LOV-1 and how ECM may be important for the structure of the cilium. These studies also advance the understanding and expand the options for treatment of ADPKD and other human ciliopathies.

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Peptide fragments of BDNF retain their bioactivity after immobilization to collagen: development of a new biomaterial for TBI therapy

Lowe, Christopher J; Shreiber, David, I

Traumatic brain injury (TBI) is characterized by an initial physical trauma followed by an inflammatory secondary injury cascade, which result in neuronal damage and death. Research efforts to protect healthy tissue and promote regeneration following injury have included the delivery of neurotrophic factors and cell transplantation. Brain derived neurotrophic factor (BDNF) can provide neuroprotection and improve neuron survival following injury. BDNF can also positively influence the survival and differentiation of neural stem cells (NSCs), which have been shown to be a therapeutically relevant cell source for transplantation, but suffer from poor viability and engraftment. Despite these advantages, the therapeutic application of BDNF is limited by high costs and rapid clearance in vivo. Recently, short peptides that mimic the activity of native BDNF have been identified. We have successfully coupled one of these peptides, IKRG, to type-I collagen in an effort to develop a neuroprotective hydrogel that also enhances the transplant efficacy of NSCs. In this work, we compared the bioactivity of our peptide grafted gels to soluble peptide and full length BDNF. Cerebellar granule neurons (CGNs) cultured on IKRG hydrogels demonstrated longer neurite outgrowth than unmodified collagen hydrogels and promoted growth similar to soluble BDNF. NSCs cultured on IKRG peptide-grafted collagen hydrogels exhibited similar viability to conditions where soluble peptide or BDNF were added to the culture media. Ongoing work will assess the ability of this hydrogel system to support neuronal differentiation of NSCs and preserve neuronal viability using in vitro models of secondary brain injury such as glutamate.

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Abstract

Lactobacilli Inactivate Chlamydia Trachomatis and Neisseria Gonorrhoeae through Lactic Acid

Gong, Z., Wu, X., Galkowski, D., Jarvis, G., Fan, H., Desai, M.

Lactobacillus species dominate the microbiome in the lower genital tract of most reproductive-age women. Producing lactic acid and H₂O₂, lactobacilli are believed to play an important role in prevention of colonization and growth of pathogens. However, how lactobacilli interact with Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (GC), leading bacterial pathogens of sexually transmitted infections (STI), has not been studied. Here, we demonstrate the inactivation of both CT and GC through culture-media conditioned by Lactobacillus species that dominate the human vaginal microbiome. Lactobacillus still cultures produced LA, leading to time- and concentration-dependent killing of CT and GC. Neutralization of the acidic media completely reversed killing. The H₂O₂ concentrations in the still cultures were found to be comparable to those in the cervicovaginal fluid, but insufficient to inactivate CT and GC. Increasing H₂O₂ production from lactobacilli resulted in decreased LA production, compromising antimicrobial activities. We noticed two significant differences in LA-mediated killing between CT and GC. First, compared to CT, GC is significantly more susceptible to LA. Second, arginine-induced acid resistance was found in GC but not CT. We conclude: 1) LA, but not H₂O₂, is both required and sufficient for the antichlamydial and antigonococcal activities of lactobacilli; 2) differential LA susceptibility may partially account for higher clinical CT rates; 3) while the basic and arginine-rich properties of semen protect all STI-pathogens by neutralizing LA, semen also protects GC through a neutralization-independent mechanism; and 4) lowering the vaginal pH by engineering the vaginal microbiome may make women less susceptible to STIs.
Disruptions to the miRNA regulatory pathway may cause an increased rate of schizophrenia in individuals with 22q11.2DS.

W. Manley, S. Siecinski, S. Ryan, L.M. Brzustowicz

Schizophrenia is a complex and poorly understood disease caused by the interplay of many environmental and genetic factors. The 22q11.2 deletion syndrome (DS) is a disorder that is caused by the microdeletion of part of chromosome 22 leading to a 25-fold greater chance of developing schizophrenia in affected individuals versus the general population. The missing 22q11.2 region contains DGCR8, which is required for the initial step of miRNA biogenesis. However, the 22q11.2 deletion itself is not directly the cause of schizophrenia, since 75% of individuals with this deletion do not develop the disease. We hypothesize that the 22q11.2 deletion increases the risk of schizophrenia through alterations in miRNA regulatory networks via depletion of several miRNAs. This may serve as a protective buffer against the accumulation of deleterious mutations at other schizophrenia risk loci.

We have derived human neural stem cells (NSCs) from individuals with the 22q11.2 deletion. The NSCs were generated from iPSCs using Neural Induction Media (Life Technologies). In order to ensure the presence of the 22q11.2 deletion, a FISH probe for 22q11.2 (TUPLE) was used (Cell Line Genetics). Also, the levels of DGCR8 gene expression were quantified using the 7900HT Fast Real-Time PCR System (Applied Biosystems) along with Taqman Gene Expression Assays to ensure DGCR8 reduction in 22q11.2 DS NSCs relative to otherwise healthy control NSCs. We have begun to characterize disruptions to the miRNA regulatory network in the NSC lines using Taqman Array Human Microarray Cards Version 3.0.

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Mechanistic Studies of RIG-I Activation and Ligand Selection.


RIG-I is a cytosolic innate immune receptor triggering interferon signaling in response to viral RNAs. It belongs to the DEAD-box family of RNA helicases and exhibits RNA binding and ATPase activities, which are both essential for initiating interferon signaling. RIG-I maintains an ‘auto-inhibited’ state mediated by an extensive CARD2-Hel2i interface, wherein the Hel2i (Helicase 2 insertion) domain sequesters the second CARD which is the effector signaling domain of RIG-I. Our work was aimed at understanding how RIG-I differentiates between self and non-self RNAs and contributions of individual domains in RNA selectivity. We created a panel of RNA ligands resembling self and viral RNAs and characterized the biochemical parameters ($K_d$, $k_{cat}$, $K_m$, $K_{d,app}$) of their complexes with wt (wild type) RIG-I as well as RIG-I mutants and individual RIG-I domains using radiometric and fluorescence based assays. We also probed the signaling potency of these different RNA modifications using cell-based reporter luciferase assays.

We show that deleting the CARD domains or mutating the CARD2-Hel2i interface leads to significant defects in RNA selectivity. We also show that the signaling activity of the panel of RNA ligands correlates with their respective ‘selectivity factor’ ($k_{cat}/K_{d,app}$). In conclusion, we provide a comprehensive biochemical basis for ligand selection by RIG-I and propose that the biochemical parameter ‘$k_{cat}/K_{d,app}$’ can be used to estimate the signaling activity of any RNA ligand. We also propose a novel role for the CARD2-Hel2i interface in acting as a ‘gate’, allowing only viral RNAs to form productive signaling complexes with RIG-I.

(This work was supported by NIH grants GM55310 to S.S.P., AI080659 to J.M and GM111959 to J.M and S.S.P.)

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Synergistic Anti-tumor Activity of the Notch Gamma Secretase Inhibitor BMS-906024 and Paclitaxel in the Treatment of Lung Adenocarcinoma


Notch signaling is aberrantly activated in approximately one third of non-small cell lung cancer (NSCLC) cases and is associated with poor overall survival among NSCLC patients whose tumors are wildtype for TP53. We set out to evaluate the combination of Notch-targeted therapy with front-line chemotherapy as an effective treatment for NSCLC. To target Notch, we utilized the gamma secretase inhibitor (GSI) BMS-906024, which inhibits Notch activation. Human cell lines representing the major genetic subtypes of lung cancer, most of which were derived from adenocarcinomas, underwent MTS drug synergy assays consisting of treatment with BMS-906024, cisplatin or paclitaxel, or the combination of GSI and chemotherapy. Analysis of the drug effects with CalcuSyn yielded Combination Index (CI) values, in which a CI of 0.5 or less was considered as strong synergism for the drug combination. We found that there were significantly lower CI values for the GSI BMS-906024 combined with paclitaxel than with cisplatin (average CI = 0.54 vs 0.85, respectively; \( P = 0.001 \)). We then grouped the cell lines by major genetic subtype (wildtype versus mutant or null for EGFR, Kras or TP53). The synergy between BMS-906024 and paclitaxel was significantly greater in Kras-wildtype than Kras-mutant cells (average CI = 0.39 vs 0.68, respectively; \( P = 0.009 \)), while there was no correlation with EGFR or TP53 status. These results are a step toward identification of potential biomarkers that could be used to predict patient response to Notch-targeted therapy, which could have a positive impact on the care of lung adenocarcinoma patients.

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Abstract 17

Regulation of the BRCA1-PALB2-BRCA2 DNA damage response pathway

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DNA double stand breaks (DSBs) are the most hazardous form of DNA damage which, if unrepaired or misrepaired, can lead to cell death or genome instability that drives tumorigenesis. The breast cancer tumor suppressors BRCA1 and BRCA2 play essential roles in cellular response to DSBs by enabling homologous recombination (HR)-mediated DSB repair and regulating cell cycle checkpoints. The two BRCA proteins are physically and functionally linked by a third tumor suppressor protein, PALB2 (partner and localizer of BRCA2). Interestingly, PALB2/BRCA2 binds to a region in BRCA1 that contains 14 serine/threonine residues that can be phosphorylated by ATM (Ataxia telangiectasia mutated) and/or ATR (Ataxia telangiectasia and Rad3 related). Our preliminary results have shown that knockdown of PALB2 resulted in elevated BRCA1 phosphorylation at Serine 1423 (S1423), which was also elevated in the presence of various DNA damaging agents such as Hydroxyurea, Bleomycin and Camptothecin. Yet, the HR activity of BRCA1-S1423A mutant was not impaired. Interestingly, MDA-MB-436 cells (triple negative breast cancer cells with biallelic BRCA1 mutations) stably expressing BRCA1-H1421Y, a Variant of Uncertain Significance (VUS) located immediately upstream of S1423, showed defective G2/M checkpoint activation upon IR and increased resistance towards cisplatin compared to cells expressing wild type BRCA1. Collectively, our findings underscore the functional complexity of BRCA1-PALB2/BRCA2 interaction that is not only critical for efficient repair of DSBs but also cell cycle checkpoint control during DDR. We are currently investigating the impact of the H1421Y variant on S1423 phosphorylation and the state of BRCA1 phosphorylation on other S/TQ sites in the region in the presence or absence of PALB2, as well as the functional roles of the phosphorylation events on DNA repair, checkpoint control and drug resistance. Insight derived from this study will advance our understanding of the mechanisms by which BRCA1/PALB2/BRCA2 maintain genome stability and prevent tumorigenesis.

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A Requirement for Aurora Kinase B that is Independent from Aurora Kinase C in Female Meiosis

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The Aurora kinases are conserved serine/threonine kinases that regulate chromosome segregation. Aurora Kinase B (AURKB) is expressed in mitotic cells while Aurora kinase C (AURKC) is primarily expressed in meiotic cells. A focus has been on AURKC, leaving the requirement for AURKB in meiosis unknown. To determine if AURKB is required during female meiosis or how much AURKB/C activity is required we utilized oocyte-specific Cre drivers to excise Aurkb. These mouse strains were crossed into an Aurkc⁻/⁻ background to develop a genetic allelic series expressing different copy numbers of the kinases. We compared phenotypes of WT mice to that of single knockouts and to those from mice containing 1 copy of Aurkb or Aurkc. Oocytes lacking AURKB but not AURKC had spindle morphology defects. Spindle abnormalities persisted in oocytes with a single copy of either kinase but were most severe in Aurkb⁻/⁻ Aurkc⁺⁻ suggesting a specific role for AURKB in regulating microtubules. This requirement persisted in those oocytes that progressed to metaphase of meiosis II both in vitro and in vivo. Aurkb⁺⁻ and Aurkb⁺⁺ Aurkc⁺⁻ eggs were significantly more aneuploid than eggs from Aurkc⁻/⁻ and Aurkb⁺⁺ Aurkc⁻/⁻ mice suggesting a requirement for the presence of AURKB to maintain the spindle assembly checkpoint. These data show that AURKB is not only required for female meiosis but that it has at least one function in regulating the spindle that AURKC is not capable of conducting.

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Mapping *Spirodea polynrhiza* post-transcriptional response patterns

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The *Lemnaceae* are the smallest, fastest growing, and most morphologically reduced angiosperm family, which show great potential in applications to wastewater treatment, biofuel, and animal feed production. The *Spirodea polynrhiza* genome was published last year, marking it as the representative genome of the family. We seek to characterize the post-transcriptional regulation of this plant in control conditions and in response to seven stimuli, including abiotic stress and hormone exposure. We are therefore analyzing SOLiD system small RNA libraries to identify conserved miRNA family members and novel miRNAs with miRDeep-P. We additionally prepared libraries of cleaved, uncapped mRNAs with the GMUCT 2.0 protocol. These libraries will allow us to align miRNAs with the targets they cleaved to validate miRNA function, and target gene predictions, using the sPARTA program. Finally, we are examining miRNAs that were differentially expressed, and targets that were differentially cleaved in each condition to characterize *Spirodea* post-transcriptional responses. We hope to establish the first catalog of *Spirodea* miRNAs and targets, and investigate seven post-translational response patterns with this data to assist and guide future studies of this species and family.

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We present evidence that the Warburg effect, defined by the dramatically enhanced metabolism of glucose to pyruvate, even in well-oxygenated cancer cells, is a consequence of mutations that enhance lipid biosynthesis at the expense of respiratory capacity. Specifically, mutations in either of the respiratory enzymes, pyruvate dehydrogenase (PDC) or α-ketoglutarate dehydrogenase (KGDC), switch their substrate specificity from the 3-carbon α-keto acid pyruvate, or the 5-carbon α-keto acid α-ketoglutarate, to the 4-carbon α-keto acid oxaloacetate, resulting in aberrant synthesis of malonyl-CoA, the essential precursor of all fatty acids. To our knowledge, this activity, oxaloacetate dehydrogenase (OADC), has never been identified in eukaryotic cells. The significance of our results is two-fold. First, it offers a novel perspective on the Warburg effect: the reprogramming of energy metabolism in cancer cells would include mutational impairment of respiration to meet the fatty acid requirements of rapidly dividing cells. As proof-of-principle, we have isolated suppressors of yeast acc1<sup>cS</sup> cold-sensitive mutant that are defective for lipid biosynthesis as a consequence of failure to synthesize malonyl-CoA at the restrictive temperature. These mutants also suppress sensitivity to Soraphen A, a potent inhibitor of Acc1 AcCoA carboxylase activity. Remarkably, in addition to restoring growth at 16°C and suppression Soraphen A toxicity, these spontaneous suppressors are respiratory defective as a result of the same nuclear mutation that bypasses Acc1. We conclude that mutational inhibition of either PDC or KGDC impairs respiration, while OADC activity enhances fatty acid biosynthesis by bypassing AcCoA carboxylase, the enzyme that catalyzes the rate-limiting reaction in lipid biosynthesis. We suggest that this novel OADC activity is specific to cancer cells and represents a novel target for development of chemotherapeutics.

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Evaluating the Effect of Msc1 with Kinetochore proteins on Chromosome Segregation

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The fission yeast msc1 gene was first identified as a multicopy suppressor of loss of Chk1 function. The absence of msc1 function results in chromosome loss, suggesting that Msc1 plays a critical role in maintaining genomic stability.

Interestingly, multiple copies of msc1 suppress the temperature sensitivity of cnp1-1, which encodes the centromere-specific histone H3 variant Cenp-A, a protein that is essential for equal chromosome segregation.

Mis6, Mis15, Mis16, Mis17 and Mis18 are all evolutionarily conserved kinetochore complex components, which are part of the Cenp-A recruitment pathway. When any of these kinetochore proteins are conditionally mutated, unequal chromosome segregation occurs at restrictive temperature.

By deleting msc1 or providing extra copies of the msc1 gene, we studied the impact of Msc1 on cell growth, viability, the chromosome mis-segregation phenotype and the localization of Mis proteins in the mis mutants.

We observed that Msc1 has genetic interactions with Mis6, Mis15, Mis16, Mis17, and Mis18 kinetochore complex proteins. Furthermore, we found that the localization of the Mis proteins is altered in the absence of Msc1. We speculate that Msc1 is important for the functions of kinetochore proteins, which may explain how Msc1 maintains normal chromosome segregation.

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Enhanced sulfur assimilation drives expression of the sulfur-rich seed storage proteins in maize

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Sulfate reduction from sulfate to sulfite catalyzed by the 5’-adenylylsulfate reductase (APR) serves as the major regulatory point in the sulfate assimilation pathway. Bacterial APRs can functionally substitute for the more complex plant APRs and expression of these bacterial enzymes in Arabidopsis and maize are able to enhance sulfate assimilation. However, constitutive expression of bacterial APRs in maize interfere with normal development. Therefore, we used the tissue-specific RbcS and PepC promoters to test whether compartmentalized expression of bacterial APRs would be an improved method for sulfur assimilation in maize and increase the levels of the sulfur-containing seed storage proteins, which are part of the zein multigene family, in the seeds. Four expression constructs (RbcS-EcPAPR; PepC-EcPAPR; RbcS-PaAPR; Pep-PaAPR) were tested for their utility in enhancing sulfate assimilation in maize seeds. Transgenic plants were generated that showed increased methionine-rich 10-kDa δ-zein and the cysteine-rich 16-kDa β-zein. The maize APR mediates a sulfate assimilation pathway that is more akin to that of PaAPR’s than EcPAPR’s. Surprisingly, we found that EcPAPR has a more pronounced effect on sulfur (S)-rich zein accumulation than PaAPR. Several backcrosses of the transgenic events to different inbreds exhibit differing patterns of S-rich zein accumulation, reflecting position and background effects. Indeed, one transgenic event expressing the PepC-driven EcPAPR results in an 18- and seven-fold increase of the 10-kDa δ-zein in a B73 and B101 background, respectively, relative to the null transgenic plant. This integration event would be a sufficient dominant marker to eliminate synthetic methionine supplementation in animal feed.

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Effects of the A118G Single Nucleotide Polymorphism (SNP) in mediating opioid and alcohol tolerance in human neurons

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Alcohol use disorders (AUDs) are major health issues imposing enormous economic and social burdens on society today. Although environmental and genetic factors largely contribute to alcohol and opioid dependence, the mechanisms mediating alcohol and opioid reward are poorly understood. Recent genetic studies have identified a single nucleotide polymorphism (SNP) of an adenine to a guanine at position 118 of human OPRM1, a gene encoding the mu opioid receptor (MOR), which strongly associates with AUDs and other drug use disorders. This results in a nonsynonymous substitution of asparagine (N) at residue 40 to an aspartate (D). By investigating the interaction of alcohol and opioid signaling in neurons carrying allelic variants for MOR N40 or D40, we hope to uncover the underlying functional consequences of the N40D SNP on drug reinforcement. To investigate this genetic variant in the context of human neurons, we have used multiple induced pluripotent stem (iPS) cell lines carrying either homozygous MOR N40 or D40 alleles from subjects with well-characterized drug dependence from which we derived subtype-specific neurons using the induced neuronal (iN) cell technology. Preliminary evidence suggests that following chronic MOR activation in inhibitory iNs by DAMGO ([D-Ala2, NMe-Phe4, Gly-ol5]-enkephalin), neurons carrying D40 alleles display defective re-sensitization compared to neurons carrying the N40 variant. This suggests altered MOR trafficking and signal transduction as a consequence of the OPRM1 A118G SNP. Our iPS cell-derived neurons provide the unique opportunity to dissect the cellular and molecular mechanisms of MOR activation on synapse functionality in a human system.

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Abstract 24

**Inhibition of Pak4 blocks growth of breast cancer cells.**

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The p21-activated kinase family of proteins promotes cell survival and plays an important role in cell proliferation, cell cycle regulation and cell shape determination. There are six mammalian PAK proteins which can be subdivided into two groups by sequence homology and mode of activation- Group A Paks consisting of Pak 1, 2 and 3 and Group B Paks consisting of Pak 4, 5 and 6. There is a growing list of evidence that PAK proteins are overexpressed in many cancer types, including colon, lung, and breast cancer. PAK4 is frequently overproduced in breast cancer, including Her2 positive and triple negative breast cancers, while it is expressed at low levels in normal mammary tissue. Our preliminary evidence showed that untransformed mouse mammary epithelial cells have low levels of PAK4 that become elevated when these cells are transformed by Her2 overexpression. Furthermore, PAK4 overexpressed in mammary epithelial cells leads to oncogenic transformation and tumorigenesis in mice. We hypothesize that PAK4 plays an important role in promoting mammary tumorigenesis, and that it can serve as an effective drug target for breast cancer treatment. To test this hypothesis, we have analyzed two orally available PAK4 Allosteric Modulators (PAM; KPT-8752 and KPT-9274), which reduce the steady state PAK4 protein level in cancer cells. We observed that when we treat breast cancer cells with KPT-8752, we severely block the cell growth. KPT-8752, is effective both in Her2 positive and triple negative breast cancer cells. Our results indicate that Pak4 is an important regulator of breast cancer cell growth. Experiments studying the effects of PAMs (KPT8752 and KPT9274) on tumorigenesis in animal models of breast cancer are ongoing.

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Abstract

Retrotransposon guided into arrested replication forks

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Long Terminal Repeat retrotransposable elements (LTR-RTs) are a large group of eukaryotic Transposable Elements characterized by flaking repeats in tandem orientation—the LTRs. The LTRs of these elements contains the promoter and other sequences that recruit proteins involved in the expression, replication, silencing, organization, and stability of these elements. A successful transposable element must maximize its reproductive amplification without jeopardizing its host, and several characterized LTR-RTs appear to accomplish this through the selection of integration sites away from protein coding sequences. However, despite the high relatedness of all studied LTR-RTs, a universal mechanism that explains how these parasitic elements avoid coding sequences has not been established. Through alignment of de novo integration sites of the LTR-RT Tf1 from the fission yeast Schizosaccharomyces pombe, we find a strong integration preference for events near the binding site of Sap1, a DNA-binding protein that controls the directionality of DNA replication by causing polar fork arrest. We further show that transposition efficiency is affected by a mutation that impairs replication fork arrest, and targeting to Sap1 binding sites correlates not with Sap1 binding but with their competence as replication fork barriers. We also show that the fork arresting activity of an independent factor provided in cis can increase the integration efficiency of a barrier-incompetent Sap1 binding site. We conclude that both Sap1 binding and replication fork arrest are necessary for Tf1 integration, and that these activities are separable. We propose that Sap1 guides insertion of Tf1 by tethering the intasome and blocking the progression of the replication fork, and that the Tf1 transposon uses features of arrested forks to insert into the host genome. Since fork arrest is detectable in many genomic features that recruit LTR-RT integration, such as type III promoters and heterochromatic sequences, these observations point at a universal mechanism for determination of LTR-RT tropism.

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How to specialize a cilium: MT glutamylation and tubulin composition regulate axonemal ultrastructure

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Cilia are morphology and functionally diverse microtubule (MT) based organelles that are vital for human development and health. To understand ciliary specialization, we compared two morphologically distinct cilia types in *C. elegans*: sinusoid shape cephalic male (CEM) and rod shaped amphid channel cilia. MT ultrastructure of CEM and amphid cilia was significantly different. The transition zone (TZ) consists of nine MT doublets, comprised of a 13-protofilament A-tubule and, 11-protofilament B-tubule, anchored to the membrane by proteinaceous Y-links. TZ immediately give rise to the ‘middle segment’, in which nine MT doublets devoid Y-links. Middle segment give rise to the distal segment, which is comprised of MT singlets and projects to the tip of the cilium. These singlets are the A-tubules of the MT doublets. B-tubules in MT doublets abruptly stop at the end of the middle segment. In contrast to the amphid cilium, the CEM cilium has a longer TZ and a shorter middle segment. Nine MT-doublets in the middle segment give rise to 18 MT-singlets, which arise via splitting and extension of both A- and B-tubules doublets at the end of middle segment. Similar MT-doublet splitting is observed in human spermatozoa. Some CEM distal MT singlets fuse at the distal ciliary tip, a feature that has been not described before.

We found that CEM axonemal ultrastructure is modulated MT post-translational modifications. CCPP-1 is a tubulin deglutamylase that is required for B-tubule stability. We found that the TTLL-11 glutamylase antagonizes CCPP-1 function in CEM neurons. In a *ttll-11* mutant background, distal B-tubules in the CEM cilia are stabilized as nine doublets. Combined, our results indicated that A-B tubule spans in CEM cilia are modulated by glutamylation state.

Alpha tubulin-6 (*tba-6*) is required to establish MT ultrastructure in CEM cilia. By serial section TEM analysis of *tba-6* mutants, we found that B-tubules in MT doublets fail to extend as separate singlets and terminate at the end of the middle segment. A-tubule singlets appeared unaffected. These results suggest that A- and B-tubule singlets are of different tubulin composition, and that B-tubules require *tba-6*.


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Studying Activity-Dependent Chromatin Dynamics in Primary Cortical Neuron Cultures

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Electrical activity dependent gene expression in cortical neurons is a tightly regulated process intimately involved in learning and memory. In order to quantify and characterize these rapid changes in histone modification types and gene expression we cultured E16.5 primary cortical neurons, modulated histone modifying enzymes with genetic and pharmacological techniques, and then analyzed gene expression changes by QT-PCR. A system by which reproducible gene expression increases in the activity regulated Immediate Early Gene Arc was established. Histone position and modification changes around the transcription start site of Arc showed movement of the histone proteins from the TSS towards a position upstream as well as the appearance of H3K4me1 mark on those histones. Lentiviruses containing shRNA against methyl transferases reliably knock down mRNA levels in cultured neurons. Treatment of SMCX KO mice with an inhibitor of MLL1 partially restores mRNA levels of some of the genes most affected by the loss of SMCX. Here we have demonstrated the utility of a culture system for studying activity dependent gene expression changes using a number of different pharmacological and genetic techniques.

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Sequence-specific RNAP-DNA interactions in transcription initiation and elongation: core recognition element (CRE)

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The crystal structure of the RNAP-promoter open complex indicates that RNAP core interacts with the transcription-bubble nontemplate-strand segment corresponding to positions -4 to +2 (core recognition element, CRE).

To determine whether RNAP-CRE interactions are sequence-specific, we constructed all nucleotide substitutions at each CRE position, and assessed effects on RNAP-DNA interaction in equilibrium binding experiments and off-rate experiments. The results show that RNAP exhibits specificity for T or G at CRE position -4, specificity for T at CRE position +1, and specificity for G at CRE position +2.

To identify RNAP residues that mediate specificity at CRE positions +2, +1, and -4, we constructed Ala substitutions of RNAP residues and assessed effects on RNAP-DNA interactions with promoters containing all nucleotide substitutions at CRE positions -4, +1, and +2. The results show that Ala substitution of substitution of βR371 eliminates specificity at CRE position -4, Ala substitution of βW183 eliminates specificity at CRE position +1, and Ala substitution of βR151, βD446, or βR451 eliminates specificity at CRE position +2.

To define the structural basis of specificity at CRE positions +1 and +2, we determined crystal structures of RPo derivatives containing all nucleotide substitutions at CRE positions +1 and +2. The results show that specificity at CRE positions +1 and +2 manifests itself not only in quantitative differences in binding thermodynamics and kinetics, but also in qualitative differences in structure.

In further work, we have used RNAP derivatives containing βR371A, βW183A, and βD446A substitutions as reagents to assess the functional significance of RNAP-CRE interactions. The results show that RNAP-CRE interactions affect the sequence-specificity of promoter binding and start-site selection during transcription initiation and also affect the sequence-specificity of translocation and pausing during transcription elongation.

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**Abstract 29**

The carbon decision tree of *Chlamydomonas reinhardtii*: How starchless mutant strains redirect carbon metabolism?

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Imposed nutrient starvation is a widely used tool to shift cellular metabolism for boosting microbial biomass/bioproducts. Under N starvation, the microalga *Chlamydomonas reinhardtii* switches allocation of photosynthate from protein biosynthesis to starch and lipids, and in mutants lacking starch biosynthesis genes, preferentially to lipids. To understand the molecular basis of the altered carbon partitioning, we undertook intracellular metabolite analysis using LC-MS/MS to compare the central carbon metabolites of multiple starchless and starch synthesizing strains upon N deprivation under photoautotrophic conditions. Kinetic studies of ¹³C-labeling and steady-state metabolite pool size analysis revealed an inhibition of flux through TCA cycle and an activation of gluconeogenesis as a global response to nitrogen starvation. Simultaneous measurements of adenylate nucleotides and the cellular energy charge (CEC) revealed a tight correlation between elevated CEC and the onset of gluconeogenesis, implying a possible role for ATP as an allosteric effector for switching metabolism. The starchless strains maintain high fluxes through the gluconeogenesis pathway, especially through glucose 6-phosphate (G6P). However, ¹³C measurements show this flux is redirected from G1P to 6PG via the oxidative pentose phosphate (OPP) pathway into GAP and DHAP, and ultimately into fatty acid precursors. This activation of the OPP pathway leads to a significant loss of fixed carbon as CO₂. Thus, the starchless mutant strains use the OPP pathway as a shunt to reallocate photosynthate, fated for starch, instead into lipids at the expense of reduced biomass accumulation.

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Isolation of Novel Gamma Retroviral Envelopes Derived from a Library of Peptides and Disulphide Mapping of Cysteine Bonds Within the Randomized Region.


We demonstrated that by randomizing 11 amino acids of the Receptor Binding Domain of the Feline Leukemia Virus Envelope (Env) protein, we successfully retargeted Env proteins. Here we will present new Env isolates from a new library of Envs screened in the osteosarcoma cells. A surprising new finding from this endeavor is that the retargeted Envs (AII and BV2) conferring the highest viral infectivity are frequently constrained by the presence of cysteine residues at specific locations within the randomized region. In addition, CP a previously isolate Env with the highest infectivity levels in osteosarcoma cells, also is constrained by the presence of cysteines within the randomized region. To further understand the role of these cysteines we use mutagenesis studies, binding assays and mass spectrometry.

Alanine scanning within the randomized region revealed that these cysteines are required for efficient viral infection in all three Env isolates. Simultaneous substitution of the cysteines with glutamic acid and lysine reconstitutes the viral infectivity levels for the AII Env, but not for the BV2 and CP Env proteins. Mass spectrometry studies showed that CP does form cystine bonds within the randomized region, but a significant portion of CP have variability in disulphide bonding patterns.

Taken together this data suggests that these cysteines within the randomized region of these Envs isolates may be acting quite distinct for each Env and provide an entry advantage for the virus. This study will assist in the design of third-generation Env libraries.

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YY1 Is Required For Intestinal Organogenesis; A Role For mTORC1?

Namit Kumar, Manasa Srivilli, Ansu O. Perekatt, Edward M. Bonder, Nan Gao & Michael P. Verzi

The role of metabolism in embryonic development is under-explored. In this study, we provide evidence that Ying-Yang1 (YY1) is a critical transcription factor for intestinal organogenesis, through regulation of mitochondrial function and downstream of the mTOR pathway. YY1 loss in the developing endoderm had no apparent consequences until midgestation, after which the intestine was underdeveloped with translucent gut and a villogenesis defect. Enterocyte differentiation is compromised with shorter apical brush border microvilli and decreased expression of the differentiation marker alkaline phosphatase. Transcriptome Analysis at E15.5 revealed genes with diminished expression upon YY1 loss are strongly associated with mitochondrial function, and ultrastructural analysis confirmed compromised mitochondrial membranes in the mutant intestine. Computational analysis identified that a similar gene regulatory signature occurs when the mTORC1 pathway is inhibited. Indeed mTOR inhibition in embryonic development partially phenocopied the YY1 mutant, with villus height significantly shortened. YY1 loss or mTOR inhibition each caused decreased expression of nuclear mitochondrial genes, and rapamycin treatment of YY1 mutant embryos failed to exacerbate the phenotype, suggesting that YY1 functions downstream of mTOR in promoting villogenesis and enterocyte maturation. Our study highlights the still unappreciated role of metabolic regulation during organogenesis; interestingly, the YY1-mutant transcriptome shows a strong gene expression correlation with samples of necrotizing enterocolitis, suggesting a potential link between mitochondrial dysfunction and this common pediatric pathology.

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Unraveling the structural basis of RNA recognition by RIG-I

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Retinoic acid-inducible gene-I (RIG-I), a member of the RIG-I-like receptors (RLRs) in the innate immune system, is responsible for detecting viral infection by recognizing pathogen-associated molecular pattern (PAMP) motifs. According to previous studies, RIG-I is activated by blunt-ended double-stranded RNAs with or without a 5’-triphosphate. Upon recognition of PAMP motifs, such as in viral RNAs, RIG-I triggers a downstream signaling cascade that induces type 1 interferon (IFN-alpha) expression and inflammatory cytokines to establish an antiviral state. Studies show that helicase RD organizes into a ring around dsRNA, capping one end and contacting both strands. This ring structure forms a cavity that has space for modified RNAs. Our goal is to further investigate the structural requirements for pattern recognition by RIG-I via x-ray crystallography using several physiologically relevant modified RNAs.

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Developing a System to Identify Factors That Promote Otic Neuronal Differentiation

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During inner ear development, neuronal differentiation correlates to cell cycle arrest. To study the cells cycle kinetics of otic progenitors undergoing neuronal differentiation, we employed an immortalized multipotent otic progenitor (iMOP) cell line that recapitulates development and differentiation of otic neurons. To enrich and visualize live cells stalled in G1, we utilized the fluorescence ubiquitination-based cell cycle indicator (Fucci). The Fucci “probe” is comprised of a bicistronic construct expressing a fragment of the human Cdt-1 (30/120) protein fused to monomeric Cherry (hCdt1-mCherry) and the N-terminal fragment of human Gemini (1-110) fused to monomeric Venus (hGem-mVenus). G1 cells accumulate hCdt-mCherry and emit red fluorescence while S/G2/M cells accumulate hGem-Venus, emit green fluorescence and hCdt-mCherry is degraded. The Fucci probe allows us to fluorescently identify different phases of the cell cycle. In order to develop an iMOP stable cell line that constitutively expresses the Fucci probe, we used the Tol2 transposon system. In the stable cell lines, the Fucci probe and the neomycin resistance gene are constitutively expressed. The conferred neomycin resistance allows for selection of the iMOP cells that stably integrated the Fucci construct in their genome. We successfully selected and expanded the stable iMOP cell lines. We expect that the percentages of fluorescence cells from the Fucci iMOP stable cell line will correlate to FACS sorted iMOP cells in G1, S/G2/M. In addition, we hypothesize that G1 phase arrested iMOP cells will efficiently undergo neuronal differentiation.

Research Support: American Hearing Foundation and Hearing Health Foundation

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**Abstract**

**Tet hydroxylates Methylated Cytosine in RNA and is Essential in Drosophila.**

Wang, F., Minakhina, S., Steward, R.

DNA methylation in Drosophila is controversial and recent genome-wide bisulfide sequencing did not uncover any methylated cytosine. However, Drosophila has one essential Tet gene. Drosophila Tet is homologous to the three vertebrate proteins, 5-methylcytosine (5mC) hydroxylases that catalyze the transition of 5mC to 5hmC, resulting in the elimination of the methyl mark on DNA. TET proteins have well-documented functions in the maintenance of vertebrate stem cells, and function as epigenetic regulators of gene expression and TE activity. Our whole genome bisulfide sequencing revealed no detectable 5hmC in Drosophila DNA. This result raises the possibility that Tet may also catalyze hydroxymethylation of 5mC in RNA (5hmrc). Our antibody staining suggests that 5hmrc indeed exists in Drosophila. Loss of Tet function results in strong ovary and brain abnormalities. Tet protein is predominantly seen on DNA implying that Tet may regulate transcription, RNA maturation or processing. Together our results suggest that Tet functions in a process other than DNA demethylation.

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Gsx1 and Nkx6.1 Interact with A Cis-element of Notch1 to Regulate Neural Development in Spinal Cord

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Notch1 function has been well established in the maintenance of neural stem cell identity, maturation of neurons, and fate determination of specific glial cells and neurons. However, transcriptional regulation of Notch1 itself is not fully understood. Previously, we have identified a cis-element (Notch1CR2, or CR2) in the second intron of Notch1 gene and determined that it’s active during neocortical development. We also found the regulatory activity of CR2 in the developing spinal cord and determined the spatiotemporal CR2 activity in CR2-GFP transgenic mouse. CR2-GFP activity was observed from embryonic day 9.5 to postnatal day 7, parallel to the neurogenesis in spinal cord. In a subset of ependymal cells GFP expression persists, even in the adult, suggesting CR2 is active in both embryonic and adult neural stem cells. Furthermore, we determined that a 139bp fragment within CR2 (CR2.a) is essential for the gene regulatory activity, via binding with Gsx1 and Nkx6.1. Evidences suggest deletion of the core sequence of Gsx1 or Nkx6.1 binding site, or knockdown of Gsx1, Nkx6.1 will dramatically reduce the activity of CR2.a. Together, our findings support a general role of CR2-Gsx1/Nkx6.1 interaction in regulating gene expression in neural stem/progenitor cells during CNS development.

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THE STUDY OF DRUG RESISTANCE TO MTOR INHIBITION

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As a fundamental regulator of cell growth and metabolism, the mammalian target of rapamycin (mTOR) plays a key role in cancer. mTOR signaling pathway is commonly dysregulated in human cancers and making mTOR as a promising therapeutic target. New generation of mTOR catalytic inhibitors have been developed in recent years and vigorously evaluated in early-stage clinical trials. In targeted-therapy, one of the prevailing mechanisms of drug-resistance to protein kinase inhibitors is the emergence of mutations in the drug-binding site. Based on the clinical experience in targeted therapeutics, acquired drug resistance caused by similar mutations in mTOR is likely, but none have been documented. Here we develop a novel S. cerevisiae system to screen for mutations affecting the drug sensitivity against diverse mTOR kinase inhibitors. A potential hotspot for resistant mutations has been identified, which is distinct from the “gatekeeper” residue. Through mutagenesis study, mTOR kinase activity and function were further characterized. Our results also reveal a potential chemical scaffold to overcome drug resistance. These observations provide insights for mTOR-targeted therapies and can serve as the basis for development of next generation inhibitors.

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Physiological Role of eEF2K in Regulating Neurogenesis during Mouse Prenatal Neocortex Development

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The neocortex has a role in mammalian higher cognition, sensory perception and movement. The neocortex consists of architecturally and functionally distinct subpopulations of projection neurons, which are derived from differentiating neural stem cells (NSCs) termed radial glia (RG). How dynamics of mRNA translation determine RG differentiation into distinct neuronal subpopulations is not well understood. Besides many regulatory possibilities, mRNA translation outcome is governed by the elongation step. In particular, eukaryotic elongation factor-2 Kinase (eEF2K) regulates translational elongation by phosphorylating and inhibiting its downstream substrate, elongation factor-2 (eEF2). Our data suggest that eEF2 phosphorylation dynamically changes in progressively differentiating RG and in post mitotic neurons of the developing neocortices. In addition, we found that a prenatal loss of eEF2k results in abnormal balance of subpopulations of neocortical projection neurons. These results suggest that eEF2k has an important role in neocortical development.

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Systematic Mutation Analysis of Residues Required for the Interaction Between Ricin and the Ribosomal Stalk Proteins.

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Ricin is a plant toxin that can inhibit protein synthesis by damaging the ribosome. It is a bioterrorism agent due to its easy accessibility and high toxicity. Ricin consists of two chains, ricin A chain (RTA) and ricin B chain (RTB). We previously showed that RTA binds to the ribosomal P-protein stalk and this interaction is required for the full toxicity of RTA. We identified several double arginine mutations at the interface of RTA/RTB that reduced toxicity, depurination level and binding affinity of RTA to yeast ribosomes. We carried out a systematic mutation analysis of the arginine residues at the interface and identified one arginine residue to be the most critical. Combination of multiple mutations reduced the depurination activity of RTA further, indicating that multiple arginine residues are involved in the interaction. Our data was supported by VASP-E (Volumetric Analysis of Surface Properties with Electrostatics) analysis, which showed that the identified arginine residues affect the electrostatic interactions between RTA and the conserved C-terminal P2 peptide. Computer modeling followed by site-directed mutagenesis, further showed that residues in a hydrophobic pocket near the identified arginine might be important for the interaction. Our results indicate that arginines and the hydrophobic pocket at the RTA/RTB interface may cooperate to form the cationic and hydrophobic docking surfaces of RTA, respectively. These docking surfaces are located on the opposite side of the active site cleft, suggesting that binding to the conserved C-terminal P-protein peptide may orient the active site of RTA towards its RNA substrate.

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Regulation of TRPM7 ion channel by NHERF proteins

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TRPM7 (transient receptor potential channel, subfamily melastatin, member 7) is an ion channel that plays an important role in the regulation of whole body Mg2+ homeostasis and the early development of vertebrates. However, the mechanism by which TRPM7 is regulated remains poorly understood. A yeast two-hybrid assay of a mouse kidney library using TRPM7’s COOH terminus uncovered two PDZ containing proteins as potential TRPM7 interacting proteins, NHERF3 and NHERF4. The NHERF (Na+/H+ exchange regulatory factor) protein family are PDZ domain containing proteins (NHERF1-4) that function as scaffolds for receptors, ion channels and membrane transporters. We performed GST pull-down purification assays using TRPM7 COOH-terminus fused to GST (GST-M7CTERM) to confirm the interaction of TRPM7 with NHERF3 and NHERF4 and also discovered that TRPM7 has the potential to bind to NHERF1 and NHERF2. NHERF3 contains 4 PDZ domains. Our experiments indicate that TRPM7 binds to NHERF3’s 2nd PDZ domain. TRPM7 has been shown to regulate early development in mouse as well as in Xenopus laevis embryos. To assess whether NHERF proteins may function as regulators of TRPM7 during early development we examined the temporal expression of the NHERF proteins. Our experiments reveal that the expression pattern of NHERF1 most closely resembles that of TRPM7. Based on these data, we concluded that interaction between NHERF proteins and TRPM7 might regulate the function of TRPM7 in early development.

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C. elegans Neurons Jettison Aggregated Proteins and Mitochondria Into the Extracellular Environment in Response to Proteotoxic Stress

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Toxicity of misfolded proteins and mitochondrial dysfunction are core factors that promote age-associated functional neuronal decline and neurodegenerative disease. Accordingly, neurons invest considerable cellular resources in chaperones, protein degradation, autophagy, and mitophagy to maintain proteostasis and energy/redox balance while avoiding neurotoxicity. Although these neurotoxic challenges have long been considered to be cell-intrinsic, evidence now supports that both misfolded human disease proteins and mitochondria from one neuron can appear in neighboring cells, a phenomenon proposed to promote pathology spread. Here we document a previously unknown capacity of C. elegans adult neurons to extrude large (~5µM) vesicles that include substantial amounts of cytoplasmic contents. These "exopher" vesicles can include fluorescent GFP or mCherry, loaded Dil, aggregated human proteins such as an expanded Q128 polyglutamine protein, lysosomes, and/or mitochondria. Inhibiting chaperone expression or autophagy, as well as compromising mitochondrial quality, enhances exopher prevalence revealing exopher- genesis as a response to stresses in proteostasis and organelle maintenance. In addition, exopher contents can be found in both neighboring and remote cells, and thus exophers appear to be components of a neuronal "contagion" mechanism. We propose that exophers are components of a conserved mechanism that constitutes a fundamental, but formerly unrecognized branch of neuronal proteostasis and mitochondrial quality control, which, when imbalanced, can actively contribute to spread of neurotoxic species relevant to pathogenesis in human neurodegenerative disease.