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Developing Breath-Based Diagnostics for Lung Infections Using Secondary Electrospray Ionization-Mass Spectrometry (SESI-MS)

Heather D. Bean, Jaime Jiménez-Díaz, Jiangjiang Zhu, and *Jane E. Hill*

Bacteria produce unique combinations of volatiles that can be used to identify the genus and species, and in many cases the strain or serovar. The ability to identify bacteria by their volatilomes has generated great expectations for rapid and non-invasive clinical tests that are able to diagnose and identify infections *in situ*, particularly for diagnosing lung infections via breath analysis. A few studies have demonstrated that breath volatiles can be used to diagnose infection. While promising, these studies mostly focus on detection of only one specific bacterial species, distinguishing between uninfected and infected patients. The ideal breath diagnostic, however, would be used to identify the pathogen responsible for the infection. We aim to advance breath-based diagnostics by using the volatile fingerprinting technique of SESI-MS to identify murine lung infections caused by *H. influenzae, K. pneumoniae, L. pneumophila, M. catarrhalis, P. aeruginosa , S. aureus,* and *S. pneumoniae,* to understand how the infection breathprints change over time, and how host volatiles contribute to diagnosis.

Arsenic exposure among infants in the New Hampshire Birth Cohort is lower via breastmilk than formula

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Background: Arsenic, a known toxicant, is regulated at concentrations >10 µg/l in U.S. public drinking water. However, in rural areas many residents rely on unregulated private wells for their household water. Previous studies indicate that breastmilk arsenic concentrations are low even in areas with very high groundwater arsenic, suggesting that breastfeeding may be protective against arsenic exposure relative to the use of powdered infant formulas reconstituted with household tap water.

Objective: Determine how infant consumption of breastmilk and formula contributes to exposure to arsenic during early infancy.

Methods: We estimated arsenic exposure via breastmilk and formula for a subpopulation (n=72) of the New Hampshire Birth Cohort (NHBC), a prospective cohort of >1000 mother-infant pairs who use a private, unregulated well as the household water source. During pregnancy, we collected a sample of home tap water and during the 6-week postpartum appointment we collected a sample of infant urine and 3-day food diary. We also collected a breastmilk sample from 9 mothers. All samples were analyzed for arsenic using inductively coupled plasma mass spectrometry (ICP-MS). We used regression models to evaluate potential predictors of infant urinary arsenic and estimated exposure for exclusively breastfed and exclusively formula fed infants using NHBCS data.

Results: Feeding mode was a strong, statistically significant predictor of urinary arsenic. The median concentration for infants who were mostly breastfed (0.24 µg/L) was 2-3 times lower compared to infants fed mostly formula (0.86 µg/L) and those fed a mix of formula and breastmilk (0.62 µg/L) (R^2=0.44, $p<0.0001$). Consistent with this finding, median estimated exposure was lower for exclusively breastfed (0.04 µg kg-1 day-1) compared to exclusively formula fed (0.2 µg kg-1 day-1) infants. The powdered component of formula accounted for over 60% of median estimated exposure but only 1% of maximum estimated exposure (31 µg kg-1 day-1).

Conclusions: Breastfed infants had lower exposures to arsenic compared formula fed infants as measured by urinary arsenic at approximately 6-weeks of age. The high prevalence of breastfeeding in this sample and low prevalence of elevated arsenic concentrations in home tap water resulted in relatively low arsenic exposure for our study population.

RESEARCH SLAM: Flame Retardant Exposure among Collegiate U.S. Gymnasts

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Gymnastics training facilities contain large volumes of polyurethane foam, a material that often contains additive flame retardants such as PentaBDE. While investigations of human exposure to flame retardants have focused on the general population, potentially higher than background exposures may occur in gymnasts and certain occupational groups. Our objectives were to compare PentaBDE body burden among gymnasts to the general U.S. population and characterize flame retardants levels in gym equipment, air and dust. We recruited 11 collegiate female gymnasts (ages 18−22) from one gym in the Eastern U.S. The geometric mean (GM) concentration of BDE-153 in gymnast sera (32.5 ng/g lipid) was 4−6.5 times higher than general U.S. population groups. Median concentrations of PentaBDE, 2,3,4,5-tetrabromo-ethylhexylbenzoate (TBB) and bis(2-ethylhexyl) tetrabromophthalate (TBPH) in paired handwipe samples were 2−3 times higher after practice compared to before, indicating the gymnasts contacted these flame retardants during practice. GM concentrations of PentaBDE, TBB and TBPH were 1-3 orders of magnitude higher in gym air and dust than in residences. Our findings suggest that these collegiate gymnasts experienced higher exposures to PentaBDE flame retardants compared to the general U.S. population and that gymnasts may also have increased exposure to other additive flame retardants used in polyurethane foam such as TBB and TBPH.

Exploration of Protein Labeling Strategies and Their Application in Fluorescence Resonance Energy Transfer

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Many biological activities involve signal transduction that requires conformational changes of signaling proteins. Fluorescence resonance energy transfer (FRET) is a powerful tool to study these conformational changes. The distance changes from FRET experiment provide valuable information to probe the details of these conformational rearrangements.

Our primary interest lies in understanding conformations of cytochrome *c* upon interactions with mitochondrial membrane, including the change in these interactions during apoptosis. For FRET study, the heme cofactor of cytochrome *c* could serve as an energy transfer acceptor except there are many other heme proteins in mitochondrial membrane, making it difficult to assign the observed signal changes to intramolecular interactions within cytochrome *c*. Hence, two different fluorophores, one as an energy transfer donor and the other as an acceptor for FRET, need to be installed at two deliberately chosen positions of the protein, which demands at least two different protein labeling strategies.

Three nitrophenol derivatives were synthesized for testing the following labeling strategies on model proteins: (1) to attach nitrophenol iodoacetamide on thiol of a cysteine residue; (2) to attach nitrophenol hydroxylamine on an N-terminal serine; (3) to attach a nitrophenol cadaverine on a glutamine residue within a Q-tag, a reaction catalyzed by transglutaminase enzyme. The relatively small size of this nitrophenol probe minimizes perturbations of protein structure upon installation. Nitrophenols also absorb highly in the visible region of the spectrum, which makes them suitable for energy transfer study with many different fluorophores. The reactions were optimized to achieve high labeling efficiencies. Bimane iodoacetamide and nitrophenol cadaverine were installed as donor and acceptor on two model proteins at desired positions by stepwise dual labeling for FRET study. Future work includes application of the developed labeling strategies to FRET studies on cytochrome *c* as it interacts with mitochondrial membranes from model organisms yeast and *C. elegans*.

Impact of Regional Variation in the Use of Locoregional Therapy Prior to Liver Transplantation

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Introduction: Locoregional therapy (LRT) is frequently employed for patients with hepatocellular carcinoma (HCC) in regions with longer waiting times for liver transplantation to reduce or stabilize tumor growth and preserve transplant eligibility. We explored impact of regional variation in the use of LRT on overall and disease-free survival following liver transplantation in a national sample of HCC patients.

Methods: A novel National Cancer Institute-funded database linking clinical (pre-transplant) and pathologic (post-transplant) explant reports with Organ Procurement and Transplantation Network Registry for 2,980 liver transplant recipients transplanted with standard HCC exceptions from 2002 to 2006 was analyzed to assess impact of LRT on overall and HCC-recurrence free survival. Logistic regression analysis was used to determine predictors of LRT utilization and calculate observed:expected (O:E) ratios at both the Donation Service Area and transplant regional levels. Adjusted Cox regression models were used to assess outcomes.

Results: LRT was used in 31% of patients transplanted with HCC listing exceptions. Clinical factors predictive of increased LRT utilization included pathologic tumor stage, lower Model for End-stage Liver Disease score, and Hepatitis C infection. Use of LRT increased by 12% per month of average waiting time in the Donation Service Area (Odds ratio 1.12 $p<.001$). Even after adjustment for regional waiting times and Model for End-stage Liver Disease score, LRT utilization varied substantially across Donation Service Areas and transplant regions. Post-LT mortality (HR=0.88, p=.04 (regional level); HR=0.86, p=.02 (Donation Service Are level)) was reduced for patients living in regions/Donation Service Ares with higher than expected use of LRT (O:E>1).

Conclusion: The use of Locoregional therapy prior to liver transplantation varies due to both clinical characteristics and expected waiting time for transplantation. Despite adjustment for these factors, significant regional variation persists, and HCC patients living in high utilization regions have improved outcomes after LT.

Depletion or overexpression of Condensin II subunits destabilizes the nuclear lamina in mammalian cells.

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The shape and organization of chromosomes within the nucleus are essential for proper gene expression, and the Condensin complexes organize chromosomes into dynamic chromatin. The nuclear lamina provides structure to the nuclear envelope and makes numerous contacts with condensed chromatin. Lamin mutations that weaken the nuclear lamina result in disorganization of chromatin and altered gene expression. Overexpression of Condensin II subunits in fruit flies hypercondenses chromosomes and disrupts the nuclear lamina similarly to lamin mutants. To determine if Condensin II influences lamina structure in human cells, we depleted or overexpressed Condensin II subunits in HeLa cells and monitored changes in the shape of the nuclear lamina. Depletion of either the CAP-H2 or CAP-D3 subunits of Condesin II resulted in mis-shapen nuclei, as did overexpression of CAP-H2. These observations indicate that chromatin condensation must be maintained at an optimum conformation and that either hyper- or hypocondensation destabilizes then nuclear lamina.

Investigating Chromosomal Instability in Tumor Initiating Cells

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Tumors are composed of heterogeneous populations of cells including a unique sub-population of tumor initiating cells (TICs) that initiate and sustain tumorigenesis. TICs, in both hematopoietic and solid tumors, self-renew and differentiate similar to normal stem cells, and these properties contribute to the phenotypic diversity and therapeutic resistance of tumors. Tumor heterogeneity also arises from genomic instability due to chromosomal instability (CIN). CIN is a high rate of chromosome mis-segregation that leads to random losses and gains of whole chromosomes. Overall, CIN correlates with drug resistance, metastasis, and poor patient prognosis and may contribute to tumor evolution and adaptability by influencing the properties of TICs. Although TICs have been identified in numerous types of cancer, it is currently unknown if TICs are CIN cells. Here we demonstrate that model TICs, glioma neural stem (GNS) cells, display chromosomal instability. GNS cells have elevated rates of chromosome mis-segregation, a non-diploid karotype, and a defective p53 pathway. Thus, TICs are chromosomally unstable. Previous work showed that the major cause of CIN in human tumor cells is the persistence of improper chromosome microtubule attachments during mitosis. Further, restoring proper chromosome microtubule attachment dynamics by increasing microtubule turnover through the over-expression of the mitotic kinesin-13 microtubule depolymerase MCAK rescues chromosome segregation defects in tumor cells. Using this strategy, we established GNS cells expressing MCAK or a dominant negative mutant MCAK and found that these decrease or increase chromosome mis-segregation rates, respectively. Currently, we are using orthotopic injections to test how altering the rate of chromosome mis-segregation influences tumorigenesis.

Breakthrough Drugs: What's in a name?

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Background: New FDA law helps speed approval of drugs that may be an improvement over existing treatments by labeling them as a "breakthrough" early in the drug development process and speeding the approval process. However, there is concern over confusion with the common meaning of the word "breakthrough" as a description of an important medical advance. Calling an unapproved drug a "breakthrough" may be a way to promote the product to the public or investors before it is approved.

Objective: To compare the use of the word "breakthrough" in the industry-driven and popular news before and after the new FDA law went into effect to determine if the word "breakthrough" is being used to describe medical advances or to refer to drugs approved under the new FDA law.

Methods: Semantic analysis of the meaning of the word "breakthrough" in industry-driven and popular news articles. I searched Lexis Nexus for articles containing the words "breakthrough" and "drug" for two periods: before the new law (January 9, 2011 to January 9, 2012) and after the new law (July 9, 2012 to July 9, 2013). To limit the number of articles discussing passage of the law, articles from the period immediately preceding implementation of the law were excluded (January 10, 2012 to July 8, 2012). Articles were categorized as being part of the industry-driven news or the popular news. I reviewed each use of the word "breakthrough" within the context of 10 words before and after, and categorized the use of the word as referring to a drug approved under the new FDA law, to medical advances, or some other meaning.

Results: Before the new law, there were 174 articles published that had 192 uses of the term "breakthrough," and after the law there were 268 articles with 465 uses of the term. In the industry-driven news, before the new law 85% of uses of the word "breakthrough" referred to a medical advance while 15% referred to drugs approved under the FDA law, while after the law, 46% of uses described a medical advance and 52% referred to drugs approved under the FDA law. In the popular news, before the law, 90% of uses of "breakthrough" referred to medical advances and 9% referred to drugs approved under the FDA law while after the new law 79% referred to medical advances and 20% referred to the new law. Concerning phrases that may indicate promotion of unapproved drugs included "…[a drug company] launched [drugname], a major breakthrough in the treatment of…" "…[a disease] can be treated with a breakthrough drug called [drugname]…" "…breakthrough drug [drugname] can transform their disease…"

Limitations: Semantic analysis derives the meaning of words based on the content of the phrases before and after the word, but this analysis leaves some uncertainty about the meaning of the word in the context of the whole article.

Conclusion: After the new FDA law, use of the word "breakthrough" increased. Industry-driven news and popular news continued to use the term "breakthrough" to describe medical advances and drugs approved under the FDA law, further confusing the meaning of the term.

RESEARCH SLAM: Supermassive Black Holes: Lighting Up Entire Galaxies

Kevin Hainline

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My research focuses on the effects that supermassive black holes can have on illuminating their host galaxies, in objects called "quasars." As the largest black holes swallow gas, this gas gets very hot and glows with enough light to rival that from all of the stars in the galaxy combined, from a region that's incredibly tiny compared to the rest of the galaxy (if our galaxy were the size of America, the central black hole would be the width of a human hair!). I study how far away this light can reach throughout the galaxy, and my results show that the brightest quasars can illuminate gas throughout the entirety of the galaxy.

Alzheimer's Patients' and Caregivers' Needs and Preferences for Decision Support Interventions

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Background: Alzheimer's disease affects over 5 million people (1in 8 over 65 years-old) and 15 million family caregivers. Costs exceed $300 billion/year, with 75% paid by taxpayers, primarily from institutionalized care (e.g., extended nursing home stays). Studies report that care planning reduces caregiving burden, improves quality of life, extends aging at home, and reduces costs. However, only 25-42% of people create care plans, stating that facing increasingly complex medical decisions is a primary source of anxiety, conflict, and caregiver burden. When these decisions are not discussed before cognition declines, caregivers face placing a loved one in institutional care. In over 100 randomized controlled trials, patients' decision aids have been shown to improve informed decision making and communication. However, few decision aids have been developed for the unique challenges of Alzheimer's families (e.g., declining cognition, sequential decisions, multiple decision makers, etc.). To inform the design of Alzheimer's decision aids, this project surveyed the decision support needs of Alzheimer's patients, caregivers, and care providers across the early, mid, and late stages of disease.

Methods: Based on the Population Needs Assessment Process, we administered a 14-item questionnaire to 60 care providers, 14 patients with early-stage memory problems, and 40 family caregivers across all stages. This questionnaire assessed: 1) which decisions are most difficult at each stage, 2) which decisions would benefit most from decision support interventions, 3) what medium in most preferred, and 4) what types of resources are needed. Data analysis involved tabulating the distributions of quantitative responses and content analysis of qualitative responses to identify themes, convergence, and divergence across sample subgroups.

Results: The 114 respondents were primarily female (72%) and Caucasian (98%). Patients and caregivers stated that decisions about screening and long term care were most difficult, while providers focused on decisions about end-of-life. Patients rated fewer decisions as "Difficult/Very Difficult" (10-43%) than caregivers (56- 89%) or providers (72-89%). Patients preferred brochures, videos, and worksheets available at the clinic, while caregivers preferred worksheets and websites that were at home and the senior centers. Respondents requested features that would compare options side-by-side, examples of how other families made "good" decisions, and tools to interactively create a personal summary of their information for discussion with their clinicians and estate planners.

Conclusions: The observed differences in decision support needs and preferences of patients and caregivers suggest that interactive and tailored interventions are needed across stages. A suite of Alzheimer's family decision aids may support the changing roles and goals of dementia decision making, and foster improved person-centered care planning for both the person with Alzheimer's disease and their family caregivers. Ultimately, supporting informed decision making and active care plan formation may improve caregivers self- efficacy and extend patients' care at home, reducing unwarranted institutionalization and costs.

Conserved RNA helicase FRH acts Nonenzymatically to Support the Intrinsically Disordered Neurospora clock protein FRQ

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Protein conformation dictates a great deal of protein function. A class of naturally unstructured proteins, termed Intrinsically Disordered Proteins (IDPs), demonstrates that flexibility in structure can be as important mechanistically as rigid structure. At the core of the circadian transcription/translation feedback loop in *Neurospora crassa* is the protein Frequency (FRQ), shown here shown to share many characteristics of IDPs. FRQ in turn binds to Frequency Interacting RNA Helicase (FRH), whose clock function has been assumed to relate to its predicted helicase function. However, mutational analyses reveal that the helicase function of FRH is not essential for the clock, and a region of FRH distinct from the helicase region is essential for stabilizing FRQ against rapid degradation via pathway distinct from its typical ubiquitin-mediated turnover. These data lead to the hypothesis that FRQ is an IDP and that FRH acts nonenzymatically, stabilizing FRQ to enable proper clock circuitry/function.

Murine BRAF^{V600E} cell lines from a model of malignant melanoma

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Mutant BRAF^{V600E} is found in over 50% of human melanomas and constitutively activates Mitogen Activated Protein Kinase signaling cascades and facilitates disease progression. The mutant BRAF inhibitor, Vemurafenib (Vem), leads to tumor regression and increased survival. However, clinical resistance to Vem usually develops within a year, underscoring the need for increased understanding of molecular mechanisms underlying BRAF^{V600E} and tumor progression. Mouse models of BRAF^{V600E} provide a system for studying melanoma *in vivo*, however there is no reported *in vitro* model that is sensitive to Vem and readily transplantable into a syngeneic host. We established melanoma cell lines from the conditional mouse model of metastatic melanoma:*Tyr::CreER;Braf^{CA};Pten$^{lox4\text{-}5/lox4\text{-}5}$*,which recapitulates human disease. Three 10 mm^3 tumors, from mice backcrossed onto a pure B6 background, were dissociated using bacterial collagenase and re-injected into immunocompromised (NSG) host mice. Secondary tumors from these hosts were dissociated and cultured in DMEM/F-12 advanced medium with 5% fetal calf serum and resultant lines were termed Dartmouth murine mutantmalignant melanoma (D4M) cell lines. Cultured D4M cells express high constitutive phosphorylation of ERK, consistent with BRAF^{V600E} melanoma cells. Importantly, Vem (3 μM) abrogates this phosphorylation and increases expression of the melanoma associated antigen gp100, consistent with Vem-induced antigen expression in human melanomas. Vem also inhibits D4M growth *in vitro* by 58% in 2 days compared to vehicle treated cells. D4M cells are readily transplantable and show no difference in growth rates in either NSG or syngeneic B6 mice, with tumors developing in one week. D4M cell lines provide a valuable model for studying the molecular mechanisms of malignant melanoma *in vitro*, and a novel transplantable model of melanoma progression *in vivo*.

Cyclin A regulates kinetochore microtubule attachments to ensure faithful segregation

Lily Kabeche

The most conspicuous event in the cell cycle is the progressive alignment of chromosomes in metaphase prior to their segregation in anaphase. Persistent k-MT attachment errors are the leading cause of chromosome missegregation leading to aneuploidy. This phenomenon is called chromosomal instability (CIN). Importantly, numerous kinetochore-microtubule (k-MT) attachment errors are present in early mitosis (prometaphase), even in diploid cells that faithfully segregate chromosomes. Therefore, these errors must be corrected in an efficient manner in prometaphase. However, the mechanism by which k-MT attachments are regulated in mitosis to allow for robust error correction is not fully understood. Here we show that k-MT attachments are less stable in prometaphase (average t1/2 of 1.8 ± 0.5 min for RPE-1 cells) and become more stable in metaphase (average t1/2 of 3.8 ± 0.5 min for RPE-1 cells). Similar changes in k-MT attachment stability are seen in PTK-1 and U2OS cells. k-MT attachment stability is maintained unstable regardless of time in mitosis and chromosome alignment suggesting that k-MT attachments are regulated in a coordinated fashion. This switch in k-MT attachment stability requires proteasome-dependent degradation of cyclin A in prometaphase. Persistent cyclin A expression maintains unstable k-MT attachment stability in cells with aligned chromosomes. Conversely, premature loss of cyclin A through siRNA or elongation of mitosis through Monastrol or Nocodazole leads to stable k-MT attachments in prometaphase and an increase in chromosome missegregation events. Thus, cyclin A degradation in prometaphase is necessary for a coordinated, decisive switch in k-MT attachment stability from prometaphase to metaphase. Importantly, cyclin A promotes efficient error correction in prometaphase by creating a cellular environment that promotes microtubule detachment from kinetochores to allow for faithful chromosome segregation.

Deconstructing Clozapine: Toward Medication for Alcoholism in Schizophrenia

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Introduction: Alcohol use disorder occurs commonly in patients with schizophrenia (~35%) and dramatically worsens their clinical course. While most antipsychotics do not lessen alcohol use in patients with schizophrenia, the atypical antipsychotic clozpine (CLOZ) does, although the mechanism by which this occurs is unknown. Since CLOZ's toxicity severely restricts its use, understanding its mechanism of action, as tested in this study, may lead to development of new drugs, safer than CLOZ, which could limit alcohol use in this population. We have hypothesized that CLOZ's ability to decrease alcohol drinking in patients with schizophrenia relates to its weak blockade of the dopamine (DA) D2 receptor (D2R) coupled with its potent blockade of the norepinephrine (NE) α2 receptor and its ability to release NE in the brain. CLOZ's ability to modulate glutamatergic signaling may also play a role in its ability to reduce alcohol intake.

Methods: Hamsters were acclimated to alcohol drinking and then treated chronically with medications. *Clozapine Deconstructed:* Hamsters received vehicle, CLOZ (2-4 mg/kg), raclopride (2 mg/kg) clonidine (0.16 - 0.64 mg/kg), guanfacine (0.01-1.0 mg/kg), m-NBP (0.1-0.25 mg/kg), D-serine (1.35-2.7 g/kg) either alone or in combination with CLOZ. *Clozapine Reconstructed:* Hamsters received low doses of haloperidol (0.02 mg/kg) and risperidone (0.2 mg/kg) either alone or in combination with desipramine (1-5 mg/kg). **Results:** Raclopride (potent DA D2/D3R blockade) and clonidine (non-specific NE α2 receptor agonist) both lessened the ability of CLOZ to suppress alcohol drinking whereas guanfacine (NE α2a receptor agonist) and m-NBP (NE α2c receptor agonist) did not alter CLOZ's effects on alcohol intake. D-Serine (NMDA receptor co-agonist) potentiated the alcohol intake reducing effects of CLOZ. The addition of desipramine (DMI, NE reuptake inhibitor) increased the ability of both haloperidol and risperidone to limit alcohol drinking, more than DMI alone. **Conclusions:** These data expand upon our current neurobiologic formulation of the action of CLOZ – to suggest that its ability to decrease alcohol drinking depends, in part, on its weak DA D2R blockade and its ability to block NE reuptake and potent NE α2 receptor antagonism. CLOZ also appears to modulate glutamatergic function in the brain, and this may also contribute to its effects on alcohol intake. Moreover, the ability of CLOZ to reduce alcohol drinking can be replicated by combining weak DA D2R blockade with NE reuptake inhibition. Further study of the mechanism of action of CLOZ will help us develop new pharmacotherapies to safely limit alcohol use in patients with schizophrenia.

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RESEARCH SLAM Of bugs and men - communication between bacteria and host cells

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You are mostly not you - 90% of the cells in your body are not human cells, but bacteria. We have come to understand that not only is there extensive communication between microbes of different species, but also between bacteria and the human (host) cells. While communication with the good, commensal bacteria that are essential for human survival establishes a balance that benefits both organisms, the bad, pathogenic bacteria communicate in a way that threatens our health. Their goal is to evade the human immune system and thus avoid eradication. One route of communication is through direct contact between bacteria and human cells, whereby bacteria inject proteins and other virulence factors into the host cells, which modulate their activity. Another way to deliver their cargo is through bacterial outer membrane vesicles, which can travel long distances, for example across the mucus layer that covers our airways. Chronic airway infection is a leading cause of death in many lung diseases. If we can understand better how pathogenic bacteria communicate with host cells, we can find ways to interrupt this communication and thereby reduce mortality of bacterial infections.

Mitochondria and cytochrome *c*: controlling life and death through conformation

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Mitochondria participate in crucial metabolic reactions and regulatory mechanisms. Included in these pathways is generation of ATP and controlled cell death, or apoptosis. Many disease states stem from defects in mitochondrial function and apoptotic regulation. The release of cytochrome *c* (cyt *c*) into the cytosol is a key step in the induction of apoptosis in mammals. Cyt *c* interactions with phosphoglycerolipid cardiolipin (CL) promote structural reorganization of the protein leading to increased peroxidase activity toward CL itself. During early stages of apoptosis there is a significant increase in reactive oxygen species which, coupled with the increased peroxidase activity of cyt *c*, results in oxidation of CL. Fluorescence imaging studies have concluded that cyt *c* interactions with CL-containing vesicles induce changes in membrane curvature and ultimately pore formation. Unfolding of cyt *c* aids in CL-oxidation and pore formation, the events that contribute to cyt *c* release from mitochondria. Our lab has previously focused on fluorescence resonance energy transfer (FRET) studies, where we have shown that upon cyt *c* binding of CL containing vesicles the protein becomes a heterogeneous mixture of distinct conformational states. Here we describe our efforts to further probe the cyt *c* – CL interaction utilizing native membranes from *C. elegans. C. elegans* provides an excellent platform for our studies due to its well described life cycle and the great genetic control of this organism. *C. elegans* nematodes are amenable to large scale growth, where gravid adults may be harvested for mitochondria-rich embryos. We are working toward efficient isolation and stripping of the outer mitochondrial membrane to expose inner membrane where CL is predominantly localized. In future studies we will utilize *C. elegans* strains with variations in CL content and perform in vivo studies of cyt *c* conformations. Ultimately these investigations will describe both the conformational changes of cyt *c* structure in context of native membrane compositions and their perturbations relevant in apoptosis.

Effects of Macrophage Specific ACAT1 Deficiency on the Progression of Atherosclerosis and Xanthomatosis

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Cardiovascular disease is a public health issue and continues to be a challenging epidemic in the U.S. Atherosclerosis is a high risk factor for dyslipidemic cardiovascular disease. To offer opportunities for therapeutic intervention of atherosclerosis, it is important to understand the patho-physiological pathways involved in foam cell formation which is a hallmark of early atherosclerotic lesions. Acyl-CoA:cholesterol acyltransferase 1 (ACAT1) has surfaced as a potential target for atherosclerosis treatment, likely due to its catalytic activity which involves conversion of free cholesterol into cholesterol esters. Cholesterol esters are major components of lipid droplets in macrophage foam cells. Research conducted to test whether inhibiting ACAT1 can prevent the progression of atherosclerosis in animal models resulted in conflicting opinions regarding its benefits. The controversy stems from reports of pharmacological inhibition of ACAT1 reducing the plaque size in APOE deficient mice, which contradicts the finding that inhibiting ACAT1 by genetic knock out (KO) results in no change in plaque size. To address this controversy we have established a macrophage specific ACAT1 KO/APOE deficient ($ACAT1^{-M-M}/APOE^{-/-}$) mouse line. Preliminary results show that depleting myeloid ACAT1 expression reduces: 1) aortic plaque occurrence, 2) plaque macrophage presence, and 3) lipid content in $ACAT1^{-M-M}/APOE^{-/-}$ fed an athero-diet. Unexpectedly we discovered that the $ACAT1^{-M-M}/APOE^{-/-}$ fed an athero-diet developed skin xanthomas, a phenotype also seen in global ACAT1 KO under an APOE -/- background. In addition an ELISA analysis showed $ACAT1^{-M-M}/APOE^{-/-}$ mice displayed elevated MCP-1 cytokine levels compared to APOE -/- control, which may suggest that silencing ACAT1 in macrophages may cause a pro-inflammatory response. This work supports the notion that inhibiting myeloid ACAT1 may be beneficial for preventing atherosclerosis progression. In light of our findings we also found that blocking ACAT1 in macrophages results in disruption of cellular cholesterol homeostasis which may be implicated in the formation of xanthomas. These studies are expected to provide insight into the pathological processes which govern the progression of atherosclerosis and xanthomatosis.

Too hot, too cold, too crowded: temperature and density effects on oxidative stress in a tropical lizard

Orsolya Rita Molnár, Ph.D.

Reactive oxygen species (ROS) or free radicals are highly reactive by-products of increased metabolic rate. Due to their high reactivity, they tend to damage important macromolecules, such as proteins and nucleic acids and therefore are a threat to the body's set of enzymes, RNA and DNA. In order to neutralize ROS, the body accumulates antioxidants to defend essential enzymes and DNA from being damaged. However, antioxidants are costly to acquire, and if individuals cannot afford these costs, the amount of antioxidants will be insufficient to neutralize all ROS. This leads to the state of oxidative stress (OS), when there will be more ROS in the blood than antioxidants and macromolecules will be subject to the deleterious effects of free radicals.

OS has been shown to differ between ontogenetic stages, genders, and populations of the same species, and to be connected to activity, nutritional state and reproduction. However, direct experiments on which factors change the level of OS have not been performed, hence, the aim of this project was to determine, whether two significant environmental factors: temperature and density are directly related to changes in OS.

I placed groups of males of a tropical squamate, the brown anole (*Anolis sagrei*), under suboptimal density and temperature treatments. Density treatment group experienced 'high' density, while temperature treatments were 'high' (36°C) and 'low' (20°C). I measured levels of OS before and after one week of treatment and compared measures to those taken from a control group under optimal conditions.

Analyses show that both suboptimal density and temperature treatments elevate levels of OS. Density showed a trend to increase levels of ROS, while temperature treatments both showed a significant effect, with 'low' temperature raising levels at a larger rate, than 'high' temperature.

Results indicate that environmental changes have a considerable effect on levels of OS, and can thus cause a decrease in populations' general condition due to damaged enzymes and/or DNA. These results are especially important if placed in the context of global climate change, as rapid shifts in temperature and species dispersion can cause physiological changes in populations, which leads to deterioration in the general health state of animals.

Pharmacological suppression of chromosomal instability in cancer cells

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Aneuploidy is a hallmark of solid tumors and many aneuploid tumor cells also display high rates of chromosome mis-segregation in a phenomenon termed chromosomal instability (CIN). Recent evidence shows that the persistence of errors in kinetochore-microtubule (kMT) attachments is the underlying cause of CIN in human cancer cells. For example, stabilizing kMT through depletion of kinesin-13 family members Kif2b and MCAK in human cell lines induces CIN and destabilizing kMTs by overexpression of Kif2b and MCAK suppresses CIN in human cancer cells. This establishes the principle that CIN can be suppressed by destabilizing kMTs, although the current method involves protein overexpression which has limited applicability outside of tissue culture. To develop alternative methods to suppress CIN, we performed a high throughput screen to identify small molecules that specifically modulate the microtubule depolymerizing and/or ATPase activities of kinesin-13 proteins. This screen uncovered two lead compounds (Cp10 and Cp57), which enhance kinesin-13-dependent microtubule depolymerization activity. Using cell-based assays we demonstrate that Cp10 and Cp57 are cell permeable and induce a potent cell cycle block in mitosis at high concentrations in aneuploid tumor cells without altering cell growth in diploid cells. This mitotic block is characterized by cells with bipolar spindles and abundant chromosomes at the poles during prometaphase. Interestingly, at lower concentrations, both compounds do not block cell cycle progression, but reduce the rate of lagging chromosomes in all cancer cell lines tested. We show that Cp10 destabilizes kMT attachments in metaphase, and that it can increase the correction of kMT attachment errors induced by transient nocodazole or monastrol treatment. Finally, cells overexpressing MCAK are hypersensitive to Cp10 suggesting that Cp10 directly target MCAK-induced microtubule depolymerization. These results provide the first evidence that pharmacologic strategies can be successful in suppressing CIN in human cancer cells.

A Description of a Community-Based Program's Procedure to Select an Evidence-Based Treatment for Depression

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Background: Community-based organizations infrequently implement evidence-based services for older adults. Referral Education Assistance & Prevention (REAP) is a statewide program throughout New Hampshire that has provided community-based outreach, preventative and education services to older adults. The program was tasked with adding an evidence-based treatment (EBT) for depression.

Objective: To describe the procedure used to determine the EBT for depression that would optimize existing program strengths to increase likelihood of successful implementation and sustainability for a community-based program.

Procedure: The workgroup used an iterative process to select an EBT and to develop and refine an implementation plan. Key features of this process included (a) a multidisciplinary workgroup of stakeholders comprised of state department representatives, REAP staff, and academic researchers; (b) identifying existing REAP strengths; (c) identifying logistic considerations; (d) identifying EBTs and an implementation plan that would capitalize on strengths and were compatible with logistics.

Results: The group held 8 meetings: meetings 1-4 focused on selecting an EBT and 5-8 focused on implementation planning. The group began with a breadth of options that were narrowed to those which seemed most likely to be implemented successfully and be sustainable. Members shared commitment to this initiative and experience working with older adults; however, different stakeholders performed unique roles. For example, researchers focused on evidence whereas REAP staff focused on service workflow.

Conclusions: A multi-stakeholder workgroup using an iterative decision process is an efficient way to develop an implementation plan within a community-based outreach program. Other community-based programs could benefit from replicating this process.

The role of SUPERMAN in establishing the boundary between male and female organs in the Arabidopsis flower

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The Arabidopsis flower is composed of 4 sepals and 4 petals, which are sterile, as well as 6 stamens and 2 carpels, which are the male and female organs of the flower, respectively. These organs are organized in 4 concentric rings, or whorls, and their identity is determined by a combinations of MADS box transcription factors. One of these transcription factors is APETALA3 (AP3), which promotes petal and stamen identity. Accordingly, AP3 is normally expressed in petal and stamen primordia, but excluded from the center of the flower bud, where carpels arise. This exclusion of AP3 from whorl 4 (the carpel whorl) is necessary for the establishment of neighboring male and female organs. SUPERMAN (SUP), a transcriptional repressor expressed in the inner part of whorl 3 (the stamen whorl), has long been known to be required for the establishment of the boundary between stamens and carpels, but how it performs this role remains unclear. This project aims to answer this question using live confocal imaging.

Passive transport disrupts grid cell firing patterns in the entorhinal cortex.

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Maintaining spatial orientation is a complex process that involves the coordination of multiple brain regions involved in processing selective components of spatial information. Head direction (HD) cells discharge as a function of the animal's heading orientation within their environment, similar to that of a compass. Theta rhythm is modulated by running speed in a freely moving rat, functioning like a speedometer. HD cell activity and theta rhythm converge upon the medial entorhinal cortex (MEC) and are important for the generation of grid cells, which are thought to play an important role in path integration. Previous studies have shown that HD cells are dependent upon vestibular inputs and that theta rhythm is primarily modulated by proprioception and motor efference, with less influence by vestibular or optic flow cues. If grid cells are generated from HD and theta inputs, then grid cell function should be dependent upon both vestibular and motor related self-movement cues. Here, we compared grid cell firing patterns between active and passive movement. We isolated MEC grid cells during active locomotion in a 1.2 m square chamber and then recorded them while the rat was passively transported around the chamber in a clear plastic box that sat upon four wheels. The rats therefore had access to visual, vestibular, and optic flow cues, but were deprived of normal proprioceptive and motor efference cues. During active movement we isolated grid cells, HD cells, and conjunctive grid x HD cells. During passive transport grid cells decreased firing rates, grid-like firing patterns were eliminated, firing became significantly more related to HD, but theta modulation was still present in the cells' autocorrelograms. Conjunctive grid x HD cells exhibited the same pattern of results. HD cells decreased firing rates, increased firing variability, but maintained the same level of directional representations. These results provide evidence that grid cell function is dependent upon proprioception and motor efference, presumably because of their relationship with theta rhythm. These results differ from previous findings because non-conjunctive grid cells were found to develop HD characteristics when manipulating theta via passive movement.

Whi3 as a model protein to reveal driving forces for phase transition of aggregation-prone proteins

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From yeast to mammals, RNA binding proteins with low-complexity domains have been connected to neurodegenerative disorders because they form pathological aggregates. We discovered Whi3, an RNA binding protein with a polyglutamine tract (polyQ, a low-complexity domain), forms heterogeneously localized aggregates in the filamentous fungus *Ashbya gossypii*. Such aggregates play functional roles in both cell cycle timing and cell polarity by binding to and spatially positioning specific transcripts. We hypothesize that Whi3 can form pathological aggregates when its polyQ is expanded like that in polyQ diseases. We are using Whi3 as a model protein to test the hypothesis that aggregation-prone proteins can phase transition between three states: monomer, functional aggregate and pathological aggregate, just as water molecules transition between gas, liquid and solid. With single molecule microscopy techniques, we found both RNA and polyQ domain are driving Whi3 phase transition. On-going research includes experimentally identifying other driving forces and using basic polymer and statistical physics principles to simulate protein assembly process and phase behavior. Understanding these processes will help to reveal mechanisms of cellular toxicity of pathological aggregates implicated in many currently untreatable degenerative diseases.

Redox-Driven Protonation and Ligand Switching of Cysteine-Bound Heme Proteins

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Cysteine-bound hemes are key components of many enzymes and biological sensors. Ligand switching is often linked to redox conversions of these metal centers, which is crucial for their functions and would offer valuable information for molecular design. Herein, we characterize the ligand switching process in two engineered mutants of yeast cytochrome *c* (cyt *c*, Thr78Cys/Lys79Gly and Phe82Cys), in which thiolate of Cys78 or Cys82 becomes one of the axial ligands to the ferric heme but is replaced by Met80 upon reduction. Ligand switching during oxidation and reduction reactions was investigated by stopped-flow and manual mixing techniques. Kinetics of $Co(phen)_3^{3+}$-mediated oxidation is biphasic, related to the formation and decay of the ferric Met80-bound intermediate. This intermediate converts to the final ferric thiolate-bound product with the rate constant $k_{3,\,M\text{-}C}$ of 7.3 s^{-1} and 1.7 s^{-1} for Thr78Cys/Lys79Gly and Phe82Cys, respectively. The trend in $k_{3,\,M\text{-}C}$ for the two mutants is inconsistent with their overall folding stability, suggesting that distinct conformational rearrangements may be involved. When comparing Met-to-Cys and Met-to-His switching in the Phe82X (X = Cys or His) mutants, the rate of latter process is much faster ($\geq$20 s^{-1}), suggesting that Met80 dissociation from the ferric heme is not the rate-limiting step in ligand substitution. Considering that Phe82Cys and Phe82His probably share the same conformational rearrangement during ligand switching, we propose that the differences in rates directly arise from the incoming ligand, and in particular their protonation state. Isothermal titration calorimetry (ITC) experiments were designed to identify the protonation state of the incoming His or Cys and whether deprotonation accompanies oxidation and ligand switching in these systems. Preliminary results suggest that in the pH range of 4.5 to 8.0, approximate one proton is released into buffer upon oxidation of the Cys variants but no deprotonation takes place during oxidation of Phe82His. Taken together, our studies illustrate that the position of the incoming ligand and its propensity to deprotonation reactions can dramatically modulate redox-linked ligand switching.