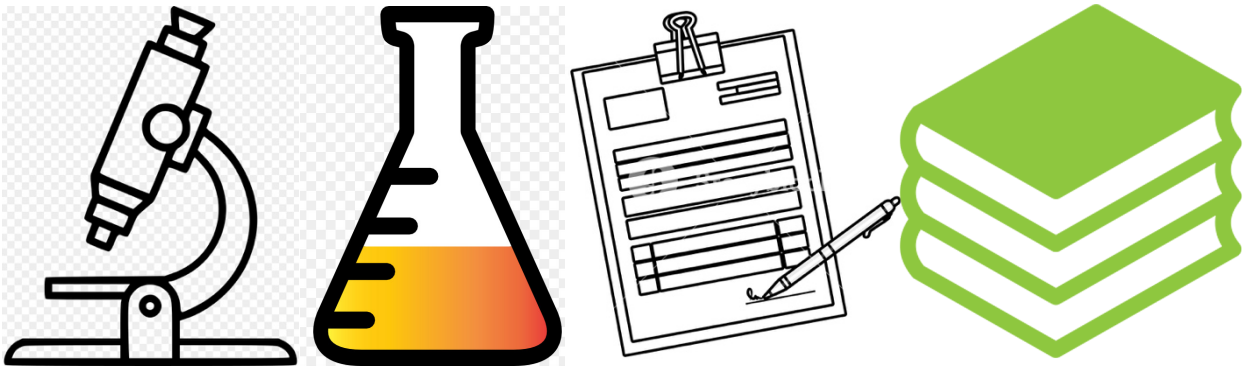
WAYNE STATE UNIVERSITY

Department of Biological Sciences



Research Retreat

2 November 2019

@ Student Center Hilberry A/B/C

Department of Biological Sciences Annual Departmental Retreat
Wayne State University Student Center
November 2, 2019
Schedule of Events

8:30-9:00 **Coffee and Pastries**
Student Center Hilberry B (230)

9:00-9:10 **Welcome – Dr. Meller (Department Chair)**
Student Center Hilberry A (240)

Featured Speakers I, Student Center Hilberry A (240)

9:10-9:30 Joy Alcedo – **Neural insulin signaling in development and stress recovery: axonal localization of an insulin-like peptide mRNA**

9:30-9:50 Qing Chen (Friedrich's Lab) – **Novel roles of the *Pax6* transcription factor gene *eyeless* in the developing *Tribolium* compound eye**

9:50-10:10 Zuzer Dhoondia (Ansari's Lab) – **RAT1 functions in co-transcriptional splicing in *Saccharomyces cerevisiae***

10:10-10:30 Chris Steiner– **The effects of habitat isolation on diversity and stability: Do intraspecific responses mirror interspecific responses?**

10:30-11:50 **Poster Session I (*odd numbered posters*)**
Student Center Hilberry C (220)

11:50-1:10 Lunch and Activities
Student Center Hilberry B (230)

1:10-2:10 **Keynote Speaker: Arthur Bradley Eisenbrey III**, **MD, PhD,** Clinical Associate Professor of Pathology, Wayne State University School of Medicine
Title: What does a (WSU) biologist do?
Student Center Hilberry A (240)

Featured Speakers II, Student Center Hilberry A (240)

2:10-2:30 Praneet Marwah (Njus's Lab) – **Dopathaizines, a novel family of dopamine oxidation products, may account for the dopaminergic selectivity of both Parkinsonism and Manganism**

2:30-2:50 P.-C. Lee – **The host Hippo pathway in microbial infection**

2:50-4:10 **Poster Session II (*even numbered posters*)**
Student Center Hilberry C (220)

4:10-4:30 **Awards/Wrap-up**
Student Center Hilberry B (230)

Featured Talk and Poster Index

Featured Talk #1

Neural insulin signaling in development and stress recovery: axonal localization of an insulin-like peptide mRNA

Rashmi Chandra, Lisa Li*, Zahabiya Husain*, Bianca Pereira*, Shashwat Mishra and Joy Alcedo
Department of Biological Sciences, Wayne State University, Detroit, MI
*Equal contribution

Aberrations in insulin or insulin-like peptide (ILP) signaling in the brain causes many neurological disorders. Here we report that mRNAs of specific ILPs are surprisingly localized to the axons of *C. elegans* during stress-induced developmental arrest, which is known as dauer. We find that axonal localization of the ILP *ins-6* mRNA correlates with faster recovery from dauer arrest. In addition, axonal *ins-6* mRNA is regulated by at least two opposing signals: one that depends on the insulin receptor DAF-2 and a kinesin-2 motor; and a second signal that acts parallel to DAF-2 and requires a kinesin-3 motor. While Golgi bodies that package nascent peptides, like ILPs, have not been previously found in *C. elegans* axons, we now show that axons of *C. elegans* dauers have increased Golgi ready to package peptides for secretion. Thus, we present a mechanism that facilitates an animal's rapid recovery from stress through axonal ILP mRNA localization.

Featured Talk #2

Novel roles of the *Pax6* transcription factor gene *eyeless* in the developing *Tribolium* compound eye

Qing Chen
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Friedrich Lab

The *Pax6* transcription factor family plays critical upstream roles in the regulation of eye development in bilaterian animals. Key insights into this process have been gained in *Drosophila*, which possesses two *Pax6* genes: *eyeless* (*ey*) and *twin of eyeless* (*toy*). Initially broadly expressed in the eye-antennal imaginal disc, *ey* is specifically required for the early specification of the *Drosophila* compound eye precursor cell population. The mature *Drosophila* compound eye consists of ~800 ommatidia. Each of them, like the vertebrate camera eye, is built from an individual lens, photoreceptors, and pigment cells. No evidence has been reported that *Drosophila Pax6* genes play roles in lens formation or pigment cell development, which, however, is the case invertebrates.

In previous work, the Friedrich lab reported evidence of a conserved role of *ey* in the specification of the compound eye of the red flour beetle *Tribolium castaneum* [1]. Surprisingly, unlike in *Drosophila*, this study also noted that *ey* knockdown induced peripheral ommatidial defects. Here I present new findings from staged *ey* knockdown experiments in the *Tribolium* pupa, which reveal that *ey* is required for the formation of the peripheral ommatidial lens cuticle and tanned non-lens cuticle that outlines every ommatidium (interommatidial cuticle). In addition, late pupal *ey* expression is necessary for the formation of the massive melanized cuticle frame (ocular diaphragm), which encloses the mature compound eye of *Tribolium*. In conclusion, I will discuss a model that reconciles the early and newly discovered late functions of *Tribolium ey* by acting as a competence factor during late cell fate acquisition and differentiation events in and around the compound eye.

Reference:

1. Yang X, Weber M, Zarinkamar N, Posnien N, Friedrich F, Wigand B, et al. Probing the Drosophila retinal determination gene network in Tribolium (II): The Pax6 genes eyeless and twin of eyeless. Dev Biol. 2009;333: 215–227.

Featured Talk #3

RAT1 functions in co-transcriptional splicing in *Saccharomyces cerevisiae*

Zuzer Dhoondia, Zahidur Arif, Marva Malik, and Athar Ansari
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Ansari Lab

Rat1 is a 5′→3′ exoribonuclease in budding yeast belonging to the XRN-family of nucleases. It is a highly conserved protein with homologs being present in fission yeast, flies, worms, mice and humans. Rat1 and its human homolog, Xrn2, have been shown to play a crucial role in poly(A)-dependent termination of transcription. Here, we report a novel role of Rat1 in splicing of pre-mRNA in budding yeast. In the absence of the functional Rat1 in the nucleus, an increase in the level of unspliced transcripts was observed in yeast cells. Inhibition of termination by inactivation of CF1A complex as well as deletion of Rat1 termination complex components, Rai 1 and Rtt103, did not affect the level of unspliced transcripts, thereby suggesting that the accumulation of unspliced mRNA in the absence of Rat1 is independent of its termination function. Increased level of unspliced transcripts observed in Rat1 mutant could be either due to the direct role of Rat1 in splicing or in degradation of unspliced transcripts. If Rat1 is degrading unspliced transcripts, then unspliced transcripts must be uncapped in the mutant strain. We therefore analyzed capping pattern of spliced and unspliced transcripts in *rat1-1* cells by RNA-IP approach. We found no difference in the capping of unspliced and spliced transcripts in the mutant. Furthermore, strand-specific TRO analysis revealed that the accumulation of unspliced transcripts in Rat1-depleted nucleus was not due to stabilization of transcripts in the absence of Rat1 catalytic activity. These results strongly suggested the involvement of Rat1 in splicing of pre-mRNA. To find out if Rat1 was playing a direct role in splicing of primary transcripts, we examined interaction of Rat1 with the introns, and its association with spliceosomal machinery. Our result shows that Rat1 exhibits a physical interaction with the intronic region of nearly one-third of intron-containing genes. Furthermore, we found interaction of Rat1 with U1 snRNP. These results strongly suggest that accumulation of unspliced transcripts in Rat1 mutant is due to a direct role of Rat1 in splicing and not due to degradation of unspliced transcripts by Rat1

Featured Talk #4

The effects of habitat isolation on diversity and stability: Do intraspecific responses mirror interspecific responses?

Christopher Steiner and Mitra Asgari
Department of Biological Sciences, Wayne State University, Detroit, MI

Of the factors known to threaten biodiversity, habitat fragmentation has had the largest impact across the broadest range of biomes in the last 50 to 100 years. Fragmentation can affect biodiversity and population stability through reduction in habitat size and reduced connectivity among patches (i.e. increasing habitat isolation). In theory, such effects can simultaneously reduce biodiversity at the interspecific and intraspecific levels, decreasing both species diversity and genetic diversity within populations. However, intraspecific and interspecific responses to habitat fragmentation have historically been considered in isolation of each other. In this study, we used field mesocosms to experimentally examine how habitat isolation (in the form of dispersal rate) alters population stability and diversity at the inter- and intraspecific levels in zooplankton assemblages. Mesocosms were inoculated with six species of zooplankton common in ponds in the region. Five taxa were stocked from isogenic lab cultures. One taxon, Daphnia pulex, was stocked with 20 clones, all identifiable using microsatellite markers allowing us to track genetic composition. We imposed three dispersal treatments in which target tanks experienced either no dispersal or a low and high rate of dispersal of plankton from neighbor tanks. We observed strong effects of increasing dispersal at both the intra- and interspecific levels. As predicted, increasing dispersal increased local species diversity and enhanced population stability (reducing temporal variation in population abundances). These effects were mirrored at the intraspecific level in *Daphnia pulex*; clonal diversity was enhanced by dispersal and temporal variability of clone abundances through time was reduced.

Featured Talk #5

Dopathaizines, a novel family of dopamine oxidation products, may account for the dopaminergic selectivity of both Parkinsonism and Manganism

Praneet Kaur Marwah, Muhammad Qureshi and David Njus
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Njus Lab

Parkinson's disease and manganism cause similar symptoms because both selectively target dopaminergic neurons. We have discovered a novel class of compounds called dopathiazines that may contribute to the dopaminergic selectivity of both conditions. Dopathiazines are formed when cysteinyl-dopamine, the principal oxidation product of dopamine in vivo, is exposed to hypochlorite. Hypochlorite is produced by the enzyme myeloperoxidase, which is reportedly elevated in Parkinson's disease. We report here two variants of dopathiazines (DTM1 and DTM2) that are involved in the movement disorders associated with chronic manganese poisoning or manganism, and one dopathiazine (DTP) implicated in Parkinson's disease. We discovered that the two-equivalent redox cycling of DTM1 and DTM2 is greatly amplified by $MnCl_2$, suggesting that manganese exposure exacerbates dopathiazine-mediated oxidative stress. Mn at micromolar concentrations accelerates the reoxidation of DTM1 and DTM2 reduced by dithiothreitol, H_2 or NADH and NADH-quinone oxidoreductase (NQO1). Other metal ions including Cu, Fe, Co, and Zn do not have this effect. The other dopathiazine, DTP, is more toxic to a dopaminergic cell line than DTM1 and DTM2, and its redox cycling is not stimulated by $MnCl_2$, indicating that it may independently play a role in dopaminergic degeneration in Parkinson's disease. We suggest that dopathiazines may occur naturally at low concentrations in the substantia nigra. Their deleterious actions may be elicited by complexing to Mn causing manganism or by an abnormal increase in concentration contributing to Parkinson's disease. Taurine, which scavenges hypochlorite, prevents the formation of dopathiazines and hence can be potentially therapeutic to both disorders.

Featured Talk #6

The host Hippo pathway in microbial infection

Pei-Chung Lee
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Pathogens and their hosts are in a constant arms race. The host-pathogen interactions is a complex process. To successfully infect their hosts, many bacterial pathogens use effector proteins to subvert host responses. By studying the effector kinase, LegK7, encoded by the intracellular pathogen, *Legionella pneumophila*, we discovered that the highly conserved Hippo pathway is a new host pathway targeted by *L. pneumophila* during infection. LegK7 directly phosphorylates the key scaffold protein MOB1 in the host Hippo pathway, and phosphorylated MOB1 functions as an allosteric activator for LegK7 and facilitates downstream substrate recruitment. By manipulating the host Hippo pathway, *L. pneumophila* alters the host responses to create a favorable environment for intracellular replication, highlighting the novel role of the host Hippo pathway, in addition to its well-established roles in development and cancers, in microbial infection.

Poster #1

Exploring the Global Determinants of Bacterial mRNA Decay

James R. Aretakis and Jared Schrader
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Schrader Lab

In eukaryotes global mRNA half-lives are strongly influenced by translation elongation[1] and by the rate of translation initiation[2]. In bacteria it is unclear what mRNA features influence global mRNA half-lives. In addition, phase-separated BR-bodies, analogous to eukaryotic P-bodies, contribute to mRNA degradation by stimulating the rate of mRNA decay[3,4]. Here we measure the genome-wide mRNA decay rates in *Caulobacter crescentus*. Then using this mRNA dataset, we investigate what features control mRNA degradation in bacteria. The strongest correlation (R= 0.49) we found with mRNA half-life is codon usage, suggesting translation elongation is a dominant factor. Translation efficiency provides the next strongest correlation (R= 0.30), and GC content (R= -0.18) and mRNA length (R= -0.06), which are associated with BR-body enrichment, are inversely correlated. As translation efficiency is a measure of both translation initiation and translation elongation, we are using synthetic mRNAs to separate and vary these two translation phases independently to determine which mRNA translation phase has the largest influence on mRNA degradation. Furthermore, we will follow up by probing the mechanisms by which translating ribosomes can either stabilize or destabilize mRNAs. Ultimately, this information will allow us to compare how mRNA decay is controlled between eukaryotic and bacterial cells.

References:

[1]Presnyak, Vladimir, et al. "Codon optimality is a major determinant of mRNA stability." Cell 160.6 (2015): 1111-1124.

[2]Chan, Leon Y., et al. "Non-invasive measurement of mRNA decay reveals translation initiation as the major determinant of mRNA stability." eLIFE 7 (2018): e32536.

[3]Al-Husini, Nadra, et al. "α-Proteobacterial RNA Degradosomes Assemble Liquid-Liquid Phase-Separated RNP Bodies." Molecular cell 71.6 (2018): 1027-1039.

[4]Al-Husini, Nadra, et al. "BR-bodies provide selectively permeable condensates that stimulate mRNA decay and prevent release of decay intermediates." bioRxiv (2019): 690628.

Poster #2

A Network of Signaling Systems Constrains MrpC Accumulation During *Myxococcus xanthus* Development

Shelby Shearer[1], Bongsoo Lee[2], Andreas Schramm[2], Penelope I Higgs[1]
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[2]Dept. of Ecophysiology, Max Planck Institute, Marburg, Germany
Higgs Lab

The *Myxococcus xanthus* developmental program is controlled by the Esp, Red, and TodK signaling systems. Deletions of each of these signaling systems leads to earlier accumulation of MrpC, a major transcription factor necessary to induce fruiting body formation and sporulation. Interestingly, simultaneous deletion of all three signaling pathways leads to a synergistic effect on MrpC accumulation suggesting undiscovered regulatory links between Esp, Red, and TodK. To explore these connections, we are examining how the levels of MrpC, Esp, Red, and TodK are altered in D*mrpC*, Desp, Dred, and D*todK* mutants. A model for the regulatory network controlling MrpC accumulation will be presented.

Poster #3

Regulation of Inositol Biosynthesis by MIPS Phosphorylation

Kendall Case
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Greenberg Lab

Myo-inositol is an essential six-carbon sugar found in great abundance in the brain. It is the precursor for phospholipids including phosphatidylinositol, which plays pivotal roles in many cellular regulatory systems. Perturbation of inositol homeostasis leads to a wide range of neurological and psychiatric illnesses. The mood stabilizing drugs lithium and valproate (VPA), used in the treatment of bipolar disorder, are hypothesized to inhibit inositol biosynthesis as their mechanism of action. Inositol is synthesized by converting glucose-6-phosphate to inositol-3-phosphate by the rate limiting enzyme, *myo*-inositol phosphate synthase (MIPS; encoded by INO1), which is then dephosphorylated by inositol monophosphatase (IMPase).

Previous studies in our lab have determined that (1) the anticonvulsant and mood stabilizing drug, VPA, indirectly inhibits MIPS; (2) MIPS is a phosphoprotein, and (3) phosphomimetic mutants of three sites reduced enzymatic activity, and yeast cells expressing a double phosphodeficient mutant (S184A/S374A) has a growth advantage and reduced sensitivity to VPA. Recently, we have shown that VPA leads to in an increase in phosphorylation within 2 min of treatment.

To further characterize the mechanism by which VPA leads to MIPS phosphorylation, we are utilizing the biotin identification (BioID) protein-protein interaction screen. In this analysis, MIPS was fused to a promiscuous biotin ligase (BioID2), which tags interacting proteins. Tagged proteins were purified and identified by MS proteomic analysis, revealing kinases that potentially phosphorylate MIPS. Identification of this kinase will link cellular regulatory mechanisms to inositol biosynthesis.

Poster #4

Valproate activates endoplasmic reticulum stress elements in human embryonic kidney cells

Mahmoud Suliman[1], Iris D. Zelnik[2], Michael W. Schmidtke[1], Anthony H. Futerman[2], Miriam L. Greenberg[1]
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Greenberg Lab

Bipolar disorder is a chronic mood disorder characterized by recurring cycles of mania and depression. It affects 2% of the population worldwide and is associated with a high rate of suicide. VPA is among the main drugs that are used to treat bipolar disorder. However, the molecular mechanism by which VPA exerts its therapeutic effect is not fully understood, and it has several side effects, such as weight gain, hair loss, teratogenicity, and hepatoxicity. Therefore, determining the therapeutic mechanism of VPA will identify specific targets for drug development that avoid the harmful side effects. Previous studies in our lab using yeast as a model organism demonstrated that VPA-mediated inositol depletion activates the unfolded protein response (UPR) by up-regulating ceramide synthesis. This is significant because deficiency in UPR activation was demonstrated in lymphocytes from bipolar disorder patients. This study tests the hypothesis that VPA exerts its mechanism of action by activating the UPR pathway. We demonstrate that VPA activates endoplasmic reticulum stress elements ERSE-I and ERSE-II and increases the protein levels of the abundant endoplasmic reticulum chaperone GRP78 in human embryonic kidney (HEK293T) cells. Furthermore, lipidomic analysis of HEK293T cells treated with VPA identified an increase in certain species of ceramides and their corresponding sphingomyelins. Future experiments will determine if VPA activates the UPR pathway by increasing these species of ceramides. These findings may identify a new mechanism whereby VPA exerts its mechanism of action.

Poster #5

Drosophila dosage compensation: learning through repetition

Sudeshna Biswas and Victoria H. Meller
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Meller Lab

Dimorphic sex chromosomes regulate sex determination in *Drosophila*. Males carry one X chromosome and a gene-poor Y chromosome. The resulting hemizygosity of X-linked genes creates a lethal imbalance in gene expression ratios. This is corrected by a mechanism called dosage compensation. In flies, dosage compensation increases X-linked gene expression in males. The dosage compensation machinery in *Drosophila* is made up of proteins and one of two redundant, long non-coding RNAs, *RNA on the X 1* and *2* (*roX1* and *roX2*) that assemble into the Male Specific Lethal (MSL) complex. *X*-localization of the MSL complex is prevented by simultaneous mutations on both *roX* genes. This is lethal to males, but not females. Small interfering RNAs (siRNA) also contribute to X recognition. Hundreds of AT-rich, 359-bp satellite repeats, named 1.688^{X} repeats, are present on the X chromosome. Some of these produce siRNA. Previous studies from our lab showed that the ectopic expression of siRNA from 1.688^{3F} (at cytological position 3F) partially rescues *roX1 roX2* males and restores localization of the MSL complex, but siRNA from other repeats did not. I will determine if sequence differences in the 1.688^{X} repeats are critical, or if the construction details of siRNA-producing transgenes determine their function. To address this question, I am generating an engineered siRNA-producing construct with 1.688^{1A} sequence (from cytological position 1A) but with size, phasing and orientation identical to that of the biologically active 1.688^{3F} construct. I will analyze siRNA production from this construct and measure the biological activity for the rescue of ro*X1* ro*X2* males. If I discover that a 1.688^{1A} construct does rescue *roX1 roX2* males in this context, I will determine if the size, orientation of phasing of the cloned repeat influences expression or processing into siRNA. If the 1.688^{1A} construct fails to rescue *roX1 roX2* males, I will conclude that minor sequence differences between 1.688^{3F} and 1.688^{1A} determine biological function. This is of particular interest as the biologically active 1.688^{3F} repeat is immediately distal to *roX1* on the X chromosome. The adjacent localization of two sources of non-coding RNA that regulate the X chromosome suggests a functional arrangement.

Poster #6

Assessment of Benthic Macroinvertebrates and Primary Productivity in Streams with a Green Infrastructure Component in Detroit-Metro and Surrounding Areas

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Urbanization often negatively impacts hydrology, water quality and food webs in freshwater systems. Green Infrastructure (GI) can serve for multiple resilient purposes, such as flood reduction, providing good habitat and protection from anthropogenic disturbances in streams and lakes. The objective of this work is to determine if natural GI in urban areas can mitigate some of the impacts of urbanization, by improving water quality, increasing biodiversity of benthic macroinvertebrates and primary productivity. Natural vegetation, as a main GI type, was compared in (1) recreational parks and (2) near roads with open canopy cover in five sites of three urban watersheds in the Detroit-Metro area (Clinton, Huron and Rouge); some sites evaluated near roads include a water retention source. Chlorophyll-a (Chl-a), phaeophytin, abiotic parameters (temperature, pH, pressure, dissolved oxygen, conductivity and flow rate) and assessment of benthic macroinvertebrates were performed in duplicate. Preliminary results suggest that chl-a was different in rivers, as was phaeophytin in parks and roads. The biodiversity of benthic macroinvertebrates is similar, but the abiotic parameters showed a fairly interaction between rivers and types of sites. Although Ephemeroptera, Plecoptera, Trichoptera (EPT) and biotic tolerance (HBI) did not differ significantly, the results could indicate a fairly amount of pollution. Future samplings will be conducted until 2021 to determine how benthic macroinvertebrates can be used as bioindicators of GI sites effectiveness in the Detroit-Metro area.

KEYWORDS: *Benthic macroinvertebrates, Green Infrastructure, Primary productivity, urban areas*.

Poster #7

Frataxin (Yfh1) deficiency in cardiolipin-deficient cells leads to defective Fe-S biogenesis

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Greenberg Lab

Cardiolipin (CL) is the signature lipid of the inner mitochondrial membrane. Disorder of cardiolipin metabolism leads to Barth syndrome (BTHS), a life-threatening disease characterized by cardiomyopathy. However, the mechanism that links CL to Barth syndrome is not known. Previous studies in CL-deficient yeast cells (*crd1*Δ) demonstrated the decreased activities of Fe-S clusters cofactors, indicated as decreased succinate dehydrogenase (SDH), ubiquinol-cytochrome *c* reductase, and aconitase. Frataxin (Yfh1) is an iron storage protein that facilitates Fe-S cluster assembly. We found that mature Yfh1/Frataxin protein levels are decreased in CL-deficient yeast and mouse cells, and in lymphoblasts from BTHS patients. In addition, we found Yfh1 binds to CL *in vitro*. Ssc1 and Ssq1 are mitochondrial matrix chaperones of Hsp70 class and play sequential roles in the import and maturation of Yfh1. We found Ssc1 overexpression in yeast *crd1*Δ mutant cells rescue growth of *crd1*Δ, and *ssq1*Δ is synthetic sick with *crd1*Δ, indicating there maybe import or/and processing defects of Yfh1. We also found mRNA levels of mitochondrial processing peptidase (MPP) are decreased in *crd1*Δ mutant cells. Based on these findings, **we hypothesize that Yfh1 deficiency in CL-mutant cells leads to defective Fe-S biogenesis, and this could be caused by impaired import or/and processing defects of Yfh1.**

Poster #8

Proximity to the promoter and terminator regions regulates the transcription enhancement potential of an intron

Katherine Dwyer
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Ansari Lab

An evolutionarily conserved feature of introns is their ability to enhance expression of genes that harbor them. Introns have been shown to regulate gene expression at the transcription and post- transcription level. The general perception is that a promoter-proximal intron is most efficient in enhancing gene expression and the effect diminishes with the increase in distance from the promoter. Here we show that the intron regains its positive influence on gene expression when in proximity to the terminator. We inserted *ACT1* intron into different positions within *IMD4* and *INO1* genes. Transcription Run-On (TRO) analysis revealed that the transcription of both *IMD4* and *INO1* was maximal in constructs with a promoter-proximal intron and decreased with the increase in distance of the intron from the promoter. However, activation was partially restored when the intron was placed close to the terminator. We previously demonstrated that the promoter-proximal intron stimulates transcription by affecting promoter directionality through gene looping-mediated recruitment of termination factors in the vicinity of the promoter region. Here we show that the terminator-proximal intron also enhances promoter directionality and confers a looped gene architecture. Furthermore, we show that both the promoter and terminator-proximal introns facilitate assembly of preinitiation complex (PIC) on the promoter region. On the basis of these findings, we propose that proximity to both the promoter and the terminator regions affects the transcription regulatory potential of an intron, and the terminator-proximal intron enhances transcription by affecting both the assembly of PIC and promoter directionality.

Poster #9

SIN3 isoforms differentially regulate cell proliferation

Anindita Mitra and Lori Pile
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Pile Lab

Temporal and spatial gene expression is necessary for cell viability and organismal development. Gene activity is regulated by histone modification patterns and positioning. SIN3 is an essential transcriptional regulator that acts as a scaffolding protein for a histone deacetylase (HDAC) complex. Two major isoforms of SIN3 exist in *Drosophila*, SIN3 220 and SIN3 187, which differ in their C termini. Work from our laboratory has shown that these isoforms have distinct expression patterns and perform overlapping as well as distinct functions. Using *Drosophila* S2 cells expressing either SIN3 220 or SIN3 187, we identified isoform specific binding sites as well as differential gene expression patterns mediated by the isoforms. Analyzing gene expression patterns in cells expressing either one of the SIN3 isoforms, we noted that compared to SIN3 220, SIN3 187 expressing cells repress various cell cycle and mitochondrial maintenance genes and activate several pro-apoptotic genes. Here, we are interested in studying the regulation of cell proliferation by the SIN3 isoforms. We observed that cells expressing SIN3 187 exhibit lower cell proliferation and viability as compared to cells expressing the SIN3 220 isoform. We also determined that the SIN3 187 expressing cells have lower oxygen consumption capacity, indicating possible mitochondrial dysfunction. We are interested to further analyze the link between the differential gene expression patterns and cell physiology. Together, these studies will help us to understand why SIN3 187 expressing cells exhibit a disadvantage in terms of cell survival. Results from these studies are expected to identify molecular mechanisms used by the SIN3 isoforms to control cell viability.

Poster #10

Probing determinants of translation initiation in non-SD mRNAs

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Schrader Lab

A key step in gene expression is when ribosomes initiate mRNA at the start codon. In *E. coli*, translation initiation at the start codon is facilitated by base pairing of the 16S rRNA and a Shine-Dalgarno (SD) site in the mRNA. Surprisingly, recent genome surveys revealed that only half of bacterial genes contain SD sequences, with some bacterial species having as few as 8% of their genes encoded with upstream SD sequences[1]. To understand the mechanism(s) of non-SD translation initiation, we utilize *Caulobacter crescentus*, an α-proteobacterium that is highly adapted to initiation in leadered non-SD mRNAs (lacking SD sequence in 5' untranslated region (UTR)) and leaderless mRNAs (lacking 5' UTRs)[2]. We hypothesize that a lack in mRNA secondary structure increases the accessibility of the start AUG codon to the ribosome thereby facilitating initiation. To test this hypothesis, we used computational analysis to predict start codon regional accessibility by calculating ΔG_{unfold} across the Caulobacter genome. The ΔG_{unfold} predictions revealed that the start AUG codons are more accessible than elongating AUG codons within the body of mRNAs. Interestingly, leaderless mRNAs have a lower ΔG_{unfold} than leadered RNAs, and generation of a set of synthetic leaderless RNAs with larger ΔG_{unfold} strongly inhibits leaderless translation. Mutations in the 5′ UTR's of SD and non-SD mRNAs revealed that $\Delta_{Gunfold}$ negatively correlates with translation levels although to a lower extent. To further establish the role of mRNA structure and start codon selection we are generating large ribosome binding site (RBS) libraries across diverse mRNAs with altered secondary structures whose translation will be assayed by flow-seq[3].

References:

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Poster #11

RNA Polymerase II Plays an Active Role in the Formation of Gene Loops Through the Rpb4 Subunit

Michael J. O'Brien
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Ansari Lab

Gene loops are formed by the interaction of initiation and termination factors occupying the distal ends of a gene during transcription. RNAPII is believed to affect gene looping indirectly owing to its essential role in transcription. The results presented here, however, demonstrate a direct role of RNAPII in gene looping through the Rpb4 subunit.
3C analysis revealed that gene looping is abolished in the *rpb4* mutant. In contrast to the other looping-defective mutants, *rpb4* cells do not exhibit a transcription termination defect. RPB4 overexpression, however, rescued the transcription termination and gene looping defect of *sua7-1*, a mutant of TFIIB. Furthermore, RPB4 overexpression rescued the *ssu72-2* gene looping defect, while SSU72 overexpression restored the formation of gene loops in *rpb4* cells. Interestingly, the interaction of TFIIB with Ssu72 is compromised in *rpb4* cells. These results suggest that the TFIIB–Ssu72 interaction, which is critical for gene loop formation, is facilitated by Rpb4. We propose that Rpb4 is promoting the transfer of RNAPII from the terminator to the promoter for re-initiation of transcription through TFIIB–Ssu72 mediated gene looping.

Poster #12

The role of SIN3 in metabolism sensing and gene expression

Imad Soukar, Anindita Mitra, and Lori Pile
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Metabolic homeostasis is an important aspect of cellular physiology, and the dysregulation of metabolism is a hallmark of many cancer types. The SIN3 complex is a global transcriptional regulator that is essential for cell viability and cell cycle progression. SIN3 serves as the scaffold for the complex; it binds many proteins through multiple interaction domains. Furthermore, data from our laboratory has shown SIN3 to have a regulatory function in many metabolic pathways. The mechanism through which SIN3 senses metabolic signals and regulates metabolic genes in response, however, is unknown. We are interested in how SIN3 can sense metabolic flux in the cell and hypothesize that **SIN3 is a metabolic sensor that affects metabolism by direct gene regulation of key metabolic enzymes.**

To manipulate metabolic flux in *Drosophila* S2 cells, we used 2-deoxyglucose (2DG) to block the early steps of glycolysis. Following 2DG treatment, we were able to identify metabolic genes that are regulated by SIN3 under metabolic flux change. Additionally, we identified SIN3 interactors by immunoprecipitating SIN3 from cells treated with 2DG, followed by LC-MS/MS analysis. Interestingly, many SIN3 interactors were differentially bound to SIN3 in 2DG treated cells compared to controls, indicating that the SIN3 complex undergoes a structure configuration change when metabolic flux is altered. The LC-MS/MS studies also showed that SIN3 interactors are differentially modified, including many phosphorylation events, dependent on glucose availability. These data support our proposed model in which SIN3 senses the metabolic status of the cell and binds to some new proteins while excluding others. Uncovering this mechanism will help us to better understand how epigenetic gene regulators impact cellular metabolism.

Poster #13

Repeat-binding proteins participate in *Drosophila melanogaster* dosage compensation

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Drosophila melanogaster males carry one X and one Y chromosome, but females have two X chromosomes. To equalize expression of the X-linked genes between the sexes, males increase the transcription of X-linked genes approximately two-fold. This is mediated by the Male-Specific Lethal (MSL) complex, which modifies chromatin to elevate expression. The MSL complex first binds at Chromatin Entry Sites (CES) on the X, and then spreads into nearby active genes. CES contain a short motif that is bound by the adapter protein CLAMP. CLAMP is necessary to attract the MSL complex to the CES. However, these motifs are also found on the autosomes. These autosomal motifs also bind CLAMP but fail to recruit the MSL complex. Another factor must therefore distinguish the X from the autosomes. Observations in several species suggest that higher order chromosome organization, or location in the nucleus, contributes to X chromosome dosage compensation. Non-histone proteins that regulate these processes might thus participate in compensation. The fly X chromosome is strikingly enriched for chromosome-specific repeats. One of these, the AT-rich 1.688^{X} satellite repeats, plays a role in identifying the X. To determine if proteins that bind AT-rich regions or satellite DNA participate in X recognition I tested selected genes for X-specific or male-specific effects by RNAi knock down. I then identified genetic interactions by performing knock down in a line compromised for dosage compensation. No knock downs produced a male-specific phenotype; but knock down of the nucleosome remodeler *Imitation switch* (*ISWI*), the satellite-binding protein *D1* and *Scaffold Attachment Factor A* (*SAF-A*) enhanced lethality in males with compromised dosage compensation. In the future I will determine if these proteins localize to the 1.688^{X} repeats by ChIP. Lastly, I will use a dual luciferase reporter assay (Reem Makki, poster 14) to determine if the genes that I have identified are necessary for recruitment of compensation by the 1.688^{X} repeats or CES. I expect these studies to contribute to our understanding of how flies identify the X chromosome for compensation.

Poster #14

Dissecting the mechanism of X recognition in *Drosophila melanogaster*

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Drosophila melanogaster males have one X chromosome while females have two. This creates an imbalance in X to autosome gene dosage between the sexes. To maintain an appropriate ratio of X gene expression, male fruit flies increase transcription from X-linked genes approximately two-fold. This involves the Male Specific Lethal (MSL) complex, which is recruited to transcribed X-linked genes and modifies chromatin to increase expression. The MSL complex is thought to assemble at Chromatin Entry Sites (CES), which contain the MSL recognition Element (MRE), and then spread *in cis* to active genes in the vicinity. Since MRE sequences are present on autosomes, it is unclear how the MSL complex recognizes X-chromatin. We found that repetitive sequences that are near-exclusive to the X chromosome, the 1.688^{X} satellite repeats, promote recruitment of the MSL complex to nearby genes. The 1.688^{X} repeats do not contain MREs. Unlike CES, the 1.688^{X} repeats do not recruit the MSL complex directly. To facilitate dissection of the mechanism of recruitment, we are developing a dual-luciferase reporter to measure the ability of DNA sequences to recruit compensation. Firefly luciferase is placed on an autosome near a transgene containing the recruiting element (1.688^{X} repeats or CES). The Firefly transgene also contains sequences that limit luciferase expression to males. Recombination mediated by Hybrid Element Insertion (HEI) was used to obtain a chromosome with closely linked landing sites. A distant *Renilla* luciferase far from recruiting elements is used for normalization. The constructed Firefly luciferase reporter is functional and luciferase expression was successfully limited to males, the sex in which dosage compensation occurs. This reporter will be used in an RNAi screen to identify genes necessary for recruitment of compensation by the 1.688^{X} repeats or CES. Measuring recruitment on an autosome avoids the confounding effects of redundant X-linked elements. We expect to be able to differentiate the recruiting pathways used by different types of DNA sequences. As 1.688^{X} sequences appear to recruit through a different mechanism than the CES, we expect to identify genes not previously known to participate in X recognition.

Poster #15

A neuropeptide neuromedin U signaling system modulates *C. elegans* developmental plasticity

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Under harsh environments, the nematode *C. elegans* can enter an alternative developmental stage called dauer arrest, which are highly resistant to stress. When conditions improve, animals exit the dauer state and become reproductive adults. The switch between the reproductive state and the dauer state is regulated by specific sensory neurons and insulin receptor DAF-2 signaling. However, the mechanism(s) through which neurons and DAF-2 signaling regulate these developmental switches remain unclear.

Two of the sensory neurons that regulate entry into dauer are ASG and ADF, which respectively express neuromedin U ligands encoded by *nlp-44* and the neuromedin U receptor *nmur-1*. A loss-of-function mutation in either one of these genes had no effect on dauer entry, but loss of *nmur-1* in the *daf-2* mutant background enhances dauer entry. This suggests that NMUR-1 inhibits entry into dauer under reduced insulin signaling. Interestingly, loss of *nlp-44* in the *daf-2* mutant background has a variable phenotype on dauer entry.

nlp-44 produces three different neuromedin U-like peptides, NLP-44.1, NLP-44.2, and NLP-44.3, where NLP-44.1 binds NMUR-1, but NLP-44.2 and NLP-44.3 bind a different neuromedin U receptor, NMUR-2 in a heterologous system (V. Reale, P. Evans, Babraham Institute, Cambridge, UK). Presently, we are addressing how these neuropeptides and the two NMUR receptors act together to modulate *C. elegans* developmental plasticity in response to environmental fluctuations.

Poster #16

The Effects of Benzene Exposure in Early Life

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Benzene (C_6H_6), a volatile organic compound (VOC), is a well-known human carcinogen that is recognized to be one of the major components of air pollution. The formation of benzene is a result of heating of the fluids, creating "vaping" aerosols that are also present in e-cigarettes. The benzene formation in the e-cigarette aerosols corresponds to approximately 100 $\mu g/m^3$ (> 30 ppm), demonstrating substantial risks from benzene exposure even from the e-cigarettes. Benzene readily penetrates the body even at low concentrations such that its chronically repeated exposure poses major health risks. Recognizing its biological toxicity, the US Environmental Protection Agency (EPA) has designated benzene as one of its priority pollutants. Type 2 Diabetes (T2D) and development of insulin resistance (IR) have been associated with long-term exposure to air and traffic pollution. Mice exposed to volatile benzene at 50 ppm, develop IR and inhibition of insulin signaling in the liver and skeletal muscle, associated with inflammatory activation of nuclear factor NF-kB signaling, without the impact on body weight. Maternal diet is a major determinant of offspring health and there is strong evidence that maternal stress alters hypothalamic developmental programming and disrupts offspring energy homeostasis in rodents. In this study, we exposed pregnant and lactating C57BL/6JB female mice to benzene at 50ppm, and studied offspring metabolic phenotypes.

We show that maternal benzene exposure triggered offspring metabolic imbalance and activated hypothalamic inflammation in a sex specific manner highlighting the programming potential of hypothalamic inflammatory responses and maternal exposure to environmental stressors.

Poster #17

Influence of sex and genetics on Zebrafish behavior in novel tank test

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Anxiety is known to be influenced by sex and genetics but the mechanism that underlie these effects is not clear. Adult zebrafish are a promising model for studying the influence of sex and genetics on anxiety as they exhibit anxiety-like behaviors when introduced to a novel environment such as bottom dwelling (staying at the bottom of tank until feel safe) and thigmotaxis (swimming near the walls of tank while exploring the environment). Although this test provides the insight into the behavioral response of fish, not much is known about how variable the responses are and how sex and genetics interact to influence behavior. Additionally, zebrafish are known to display two distinct behavioral strategies (bold and shy) in response to a stressor. However, based on the anecdotal evidence we speculate that fish actually demonstrate more than two distinct coping styles. Here, we used novel tank test and recorded the swim behavior of male and female fish of four different zebrafish inbred strains. We captured three-dimensional swim patterns using Intel RealSense cameras that use an inbuilt color camera to give an image in x-y plane and stereoscopic infrared cameras to provide depth information. Tracking of the fish was done using a deep learning approach (DeepLabCut) to identify different body parts (Head, Trunk and Tail), within each frame of the video. Using this tracking data, we calculated distance from bottom, distance from center and distance travelled by fish. Our preliminary data suggests that sex and genetics influence anxiety-like behavior. For example, we observed that females in one strain demonstrate higher anxiety-like behavior whereas in another strain male fish are more anxious. Furthermore, we found that the overall distance travelled by male fish is greater than female fish during the novel tank exposure. Our future research will focus on continuing to explore whether zebrafish display different behavioral profiles beyond the bold and shy response traits, and to determine if sex and genetics influence these response traits. Overall our preliminary findings support our hypothesis and lay the groundwork for using zebrafish as research model to understand the neural and molecular basis of behavioral variations.

Poster #18

RAT1 functions in Co-transcriptional Splicing in *Saccharomyces cerevisiae*

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Rat1 is a 5′→3′ exoribonuclease in budding yeast belonging to the XRN-family of nucleases. It is a highly conserved protein with homologs being present in fission yeast, flies, worms, mice and humans. Rat1 and its human homolog, Xrn2, have been shown to play a crucial role in poly(A)-dependent termination of transcription. Here, we report a novel role of Rat1 in splicing of pre-mRNA in budding yeast. In the absence of the functional Rat1 in the nucleus, an increase in the level of unspliced transcripts was observed in yeast cells. Inhibition of termination by inactivation of CF1A complex as well as deletion of Rat1 termination complex components, Rai 1 and Rtt103, did not affect the level of unspliced transcripts, thereby suggesting that the accumulation of unspliced mRNA in the absence of Rat1 is independent of its termination function. Increased level of unspliced transcripts observed in Rat1 mutant could be either due to the direct role of Rat1 in splicing or in degradation of unspliced transcripts. If Rat1 is degrading unspliced transcripts, then unspliced transcripts must be uncapped in the mutant strain. We therefore analyzed capping pattern of spliced and unspliced transcripts in *rat1-1* cells by RNA-IP approach. We found no difference in the capping of unspliced and spliced transcripts in the mutant. Furthermore, strand-specific TRO analysis revealed that the accumulation of unspliced transcripts in Rat1-depleted nucleus was not due to stabilization of transcripts in the absence of Rat1 catalytic activity. These results strongly suggested the involvement of Rat1 in splicing of pre-mRNA. To find out if Rat1 was playing a direct role in splicing of primary transcripts, we examined interaction of Rat1 with the introns, and its association with spliceosomal machinery. Our result shows that Rat1 exhibits a physical interaction with the intronic region of nearly one-third of intron-containing genes. Furthermore, we found interaction of Rat1 with U1 snRNP. These results strongly suggest that accumulation of unspliced transcripts in Rat1 mutant is due to a direct role of Rat1 in splicing and not due to degradation of unspliced transcripts by Rat1

Poster #19

What underlies the food-type influence on oogenesis onset?

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Different aspects of an animal's life are directly influenced by the quality of its food and the same is true in the worm Caenorhabditis elegans[1]. Recently, we have shown that food quality also modulates onset of oogenesis in the worm. This requires the insulin-like peptide ins-6 signaling from the ASJ pair of taste neurons. However, these neurons express other insulin-like peptides (ILPs), which we are now testing for onset of oogenesis phenotypes in response to food quality. Moreover, because ASJ also senses the pheromones that signal population density[2], we are examining if population density has any role in oogenesis. Thus, our work will provide a better understanding of how food quality affects oogenesis.

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Poster #20

Insulin/IGF signaling and the actin-binding scaffold protein FILAMIN-2 affect *C. elegans* survival in response to different bacterial food sources

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Animal survival is influenced by food level or composition, where the effects are mediated by the perception and/or metabolism of macro- and micro-nutrients. Diet also affects insulin/IGF signaling, which is a major regulator of longevity in diverse species. The *C. elegans daf-16*/FOXO transcription factor is a downstream effector of insulin/IGF signaling and promotes longevity. To investigate the molecular mechanisms that affect diet-dependent survival, we have measured the lifespan of *daf-16* and *daf-2* insulin/insulin-like growth factor receptor mutants fed two different bacteria. *daf-2* mutants live long on both bacteria, but the effect is larger on *E. coli* CS180 than on *E. coli* OP50. In contrast, *daf-16* mutants live short on both bacteria, where the effect is again more robust on *E. coli* CS180, which suggest that insulin signaling is more active in regulating longevity on CS180 than on OP50.

What promotes the food type-dependent effects of insulin/IIS on longevity? One candidate is the actin-binding scaffold protein, *filamin-2 (fln-2)*, whose expression might be regulated by *daf-16*. Interestingly, human FILAMIN has been shown to interact with the insulin receptor. Here we show that *C. elegans fln-2* shortens lifespan on *E. coli* OP50, but not on *E. coli* CS180. This *fln-2* phenotype seems to be independent of insulin signaling, but instead involves defects in pharyngeal architecture, which depends on bacterial LPS structure. However, a loss-of-function mutation in *daf-16* unmasks a pharynx-independent *fln-2* lifespan phenotype on *E. coli* CS180, suggesting that an interaction between the two genes may affect diet-dependent survival. This also suggests that *fln-2* contributes to survival through multiple mechanisms as expected for a scaffold protein.

In mammals, FILAMIN and insulin signaling interact with toll-like receptor (TLR) pathways, which have major roles in sensing environmental cues, like bacterial LPS. Interestingly, like *daf-16*, *tol-1* TLR also masks a pharynx-independent *fln-2* lifespan phenotype on CS180. This suggests that insulin and TLR pathways work together to regulate *fln-2* activity in response to dietary bacteria. Since FILAMIN-2 is also proposed to bind to the actin cytoskeleton, our data suggest that food quality influences longevity by altering the scaffolding activities that link signaling molecules to the cytoskeleton.

Poster #21

An amino-terminal threonine/serine motif is necessary for activity of the Crp/Fnr homolog, MrpC, and for *Myxococcus xanthus* developmental robustness

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Forty to eighty percent of bacteria on earth reside in biofilms, which are encased in exopolymeric substances making them difficult for antibiotics to penetrate. Biofilms inhabit many places, including medical devices, bodies of immunocompromised patients, and pipes, causing problems in health as well as industry. *Myxococcus xanthus* is a Gram negative, non-pathogenic, soil dwelling bacterium that forms a biofilm upon starvation. Cells either 1) aggregate then sporulate inside mounds of 100,000 cells (15% of population), 2) remain dormant outside of mounds (5% of population), or 3) undergo death (80% of population). MrpC is essential to this process and is a member of the widely utilized bacterial Crp/Fnr transcription factor superfamily. MrpC contains a TTSS motif in its N-terminal extension that was previously proposed to be phosphorylated in order to control its function. Our biochemical and genetic analyses, however, revealed that the TTSS motif was not directly phosphorylated, and that MrpC was phosphorylated instead within its cNMP- and DNA-binding domains, likely under non-standard laboratory conditions. A role of MrpC as a developmental buffer was exposed through substitution analysis of the TTSS motif: a TTSS to AAAA mutant fails to aggregate and sporulate, while mutants bearing triple (TAAA or AAAS) or non-consecutive double (TASA) substitutions display variable inter- and intra-clone phenotypes. Electromobility shift assays and immunoblot data showed that TTSS motif is required for full binding of MrpC to DNA, thus allowing activation of target genes, and also likely serves as an important polar motif needed for interaction of MrpC with other proteins.

Poster #22

PAK1 Signals the Maturation of Invadopodia and Enhancement of Cancer Invasion in Response to Mechanical Stimulation

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The progression and metastasis of cancer are influenced by various mechanical forces present in the microenvironment of cancer cells. By employing an *in vitro* mechano-invasion assay, we found that mechanical stimulation leads to the maturation of invadopodia in human fibrosarcoma cells enhancing cell invasion. Invadopodia are F-actin rich protrusions that secrete proteolytic enzymes to promote the invasiveness of metastatic cancer cells and other naturally invasive cells. We determined that the mechanical response resulted from a downregulation of the integrin β3 and an activation of the actin severing protein cofilin. Previous studies have identified a pathway linking integrin β3 to the activation of cofilin through a series of signaling molecules including Rac-GTPase, PAK1 and LIMK. In this study we demonstrate that the phosphorylation and activity of p-21 activated kinase 1 (PAK1) is dependent upon integrin β3 expression levels. We find that decreased expression and activity of PAK1 in mechanically stimulated cells leads to an increase in cell invasion and maturation of invadopodium, as determined by length and proteolytic activity. Additionally, a constitutively active phosphorylation mutant of PAK1 disrupts the maturation of the invadopodium, while overexpression of a phosphorylation dead mutant enhances invadopodium maturation and cell invasiveness. Furthermore, the phenotype of the phosphorylation dead mutation of PAK1 can be overruled by the overexpression of integrin β3, suggesting that integrin β3 lies upstream of PAK1 as predicted. Our results implicate PAK1 in a signaling pathway necessary for the maturation of invadopodium and enhanced invasiveness in response to transient mechanical stimulation.

Poster #23

C. elegans stress recovery via axonal localization of the insulin-like peptide *ins-6* mRNA

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Stress responses in *C. elegans* is regulated by an insulin-like peptide (ILP) network[1]. The ILP *ins-6*, a major node in the network[1], plays a significant role in stress recovery—for example, in promoting exit from a stress-induced developmental arrest, known as dauer[2]. Intriguingly, *ins-6* mRNA becomes localized to dauer axons, which is associated with an increase in dauer recovery and depends on the activities of the insulin receptor DAF-2 and specific kinesins. Our finding of a second axonally localized ILP mRNA, *daf-28*, revealed sequence homology between the two mRNAs, which promote exit from dauer. This homology resides in the open reading frames and is absent in other mRNAs that do not promote recovery. Thus, we are now testing whether this sequence directs mRNAs to the axons, which should facilitate future live imaging of axonal mRNA transport and local translation prior to dauer exit. Because we have also found axonal Golgi in dauers, the further identification of specific sub-axonal compartmentalization of ILP mRNAs should elucidate synaptic neuron partners and a potential neural circuit that regulates stress recovery. Together our study will yield insights into mechanisms that promote optimal stress recovery, which might also be found in other animals.

References:

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Poster #24

Cardiolipin-deficient cells require NAD generated by fermentation

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Perturbation of the cardiolipin (CL) remodeling gene tafazzin leads to the X-linked disorder Barth syndrome (BTHS). In the yeast *Saccharomyces cerevisiae*, disruption of CL synthase gene *CRD1* or the tafazzin gene *TAZ1* leads to mitochondrial dysfunction, including defects in the electron transport chain (ETC) complexes. The ETC carries out oxidative phosphorylation to generate ATP, accompanied by the oxidation of NADH to NAD. Thus, the ETC is important for the maintenance of NAD/NADH redox balance in cells. NAD is an important coenzyme that is synthesized from tryptophan and aspartic acid (*de novo*) or from precursors such as niacin (salvage). The recycling of NAD from NADH is carried out mainly by the ETC and subordinately by the yeast ethanol fermentation pathway or the mammalian lactate fermentation pathway. We **hypothesized** that the ETC defects of CL mutants lead to decreased NAD levels. To replenish the NAD required by glycolysis, CL mutants upregulate the ethanol fermentation pathway. Consistent with the hypothesis, **we show that** *crd1*Δ temperature sensitivity is exacerbated if the NAD precursor niacin is absent. Supplementation of the NAD precursor nicotinamide riboside (NR) rescues the sensitivity. Under heat stress, *crd1*Δ upregulates the ethanol fermentation pathway, which seems to be important in maintaining the NAD/NADH redox balance. Moreover, supplementation of NR increases the respiration rate of BTHS mouse cardiomyocytes and is beneficial for yeast cell growth on respiration medium. This indicates a positive effect of NAD precursors on mitochondrial function. Further studies are required to determine (1) if NAD precursors will reduce fermentation and (2) how NAD precursors improve mitochondrial function in CL mutants. Understanding the regulation of NAD and the benefits of NAD precursors in CL mutants may shed light on the pathogenesis and treatment of BTHS.

Poster #25

Identification of ICP0 Sequences and Late Viral Proteins Required for the Nuclear-to-Cytoplasmic Translocation of HSV-1 ICP0

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Infected Cell Protein 0 (ICP0) is an immediate early protein of herpes simplex virus 1 (HSV-1) that counteracts cell intrinsic antiviral responses in order to enhance viral expression and replication. One unique aspect of ICP0 is its translocation between intracellular compartments at different infection stages. ICP0 is found colocalized with discrete nuclear structures termed ND10s immediately after its *de novo* synthesis. It is then released from ND10 to fill the entire nucleus as infection progresses, and later is translocated to the cytoplasm. We have previously shown that deletion of 57 amino acids from ICP0 C terminus completely blocks ICP0 translocation from nucleus to the cytoplasm in non-permissive HEL cells. Using the permissive U2OS cells, we found that late viral proteins and ICP0 C-terminus coordinate to overcome the nuclear retention of ICP0. In the present study, we investigated the role of two HSV-1 late proteins, the tegument protein VP22 and the major capsid protein VP5, in ICP0 nuclear-to-cytoplasmic translocation. We found that siRNA knockdown of VP5 did not affect the cytoplasmic localization of ICP0, whereas deletion of VP22 in viral genome led to substantial delay of the translocation. Interestingly, when VP22 and the C terminal of ICP0 were both deleted from HSV-1 genome, nuclear-to-cytoplasmic translocation of the truncated ICP0 was completely blocked, whereas ectopic expression of VP22 in a complementary cell line completely restored the cytoplasmic translocation of full-length ICP0 but only partially compensated for the translocation of the C-terminal truncated ICP0. We further analyzed ICP0 C-terminal sequence and found a potential nuclear export sequence in the last 35 amino acids. Our results indicate that a complex mechanism composed of ICP0 C-terminus and maybe multiple late viral proteins regulates the process of ICP0 nuclear-to-cytoplasmic translocation in HSV-1 infection. The biological functions of cytoplasmic ICP0 are currently under investigation.

Poster #26

The role of insulin signaling and population density in the food-type influence on oocyte maturation

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Food quality affects oocyte maturation in different animals, including the worm C. elegans. Worms fed the standard laboratory bacterial food source, *E. coli* OP50, show slower oocyte maturation than worms fed E. coli CS180. Food type similarly modulates the onset of oogenesis, which depends on insulin signaling from specific neurons. Interestingly, a strong reduction-of-function mutation in the C. elegans insulin receptor also decreases oocyte maturation, albeit in a food type-independent manner. Thus, we are currently identifying the insulin-like peptide(s) that alter oocyte maturation rates. Because insulin signaling is affected by population density[1,2], this raises the possibility that oocyte maturation is likewise subject to population density, which we are now also testing. Together our study should increase our understanding of the mechanisms that influence oocyte maturation. Considering the conservation of many processes between *C. elegans* and humans, our study could further yield therapeutic insights into infertility disorders among human females.

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Poster #27

The TodK histidine kinase and its role in cell fate segregation during the *Myxococcus xanthus* developmental program

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Myxococcus xanthus is a model organism for bacterial signaling complexity. Under nutrient limiting conditions, these bacteria enter a multicellular developmental program wherein cells follow different fates: aggregation into mounds (fruiting bodies) followed by differentiation into environmentally resistant spores, differentiation into a persister-like state termed peripheral rods, and programmed cell death. MrpC, a developmental transcriptional regulator, appears to play an important role in cell fate segregation. MrpC accumulates heterogeneously in the developing population and mis-accumulation of MrpC interferes in appropriate cell fate segregation.

TodK is a histidine kinase that appears to be necessary for regulation of MrpC accumulation. We demonstrate that a *todK* mutant over-accumulates MrpC and sporulates more quickly than the wild type, and expression of *todK* from a constitutive promoter leads to an extremely delayed developmental phenotype and reduced MrpC accumulation. To elucidate how TodK interacts in the MrpC genetic regulatory network, we examined expression of *mrpC* and its upstream activator, *mrpB,* by qRT-PCR in wild-type and various *todK* mutant strains. Based on preliminary data, we observe that expression of *mrpC* coincides with the differences in the developmental progression timing in these strains. From these data, we hypothesize that autophosphorylated TodK acts as a phosphatase to inactivate MrpB, leading to a decrease in *mrpC* expression. In contrast, unphosphorylated TodK enhances *mrpC* expression independent of MrpB, likely through disruption of MrpC negative autoregulation. We also show that MrpC is required for promoting expression of *mrpB*, revealing a positive feedback loop on MrpB. These data suggest that TodK acts to fine-tune production of MrpC in response to microscale environmental conditions.

Poster #28

Dopathaizines, a Novel Family of Dopamine Oxidation Products, May Account for the Dopaminergic Selectivity of Both Parkinsonism and Manganism

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Parkinson's disease and manganism cause similar symptoms because both selectively target dopaminergic neurons. We have discovered a novel class of compounds called dopathiazines that may contribute to the dopaminergic selectivity of both conditions. Dopathiazines are formed when cysteinyl-dopamine, the principal oxidation product of dopamine in vivo, is exposed to hypochlorite. Hypochlorite is produced by the enzyme myeloperoxidase, which is reportedly elevated in Parkinson's disease. We report here two variants of dopathiazines (DTM1 and DTM2) that are involved in the movement disorders associated with chronic manganese poisoning or manganism, and one dopathiazine (DTP) implicated in Parkinson's disease. We discovered that the two-equivalent redox cycling of DTM1 and DTM2 is greatly amplified by $MnCl_2$, suggesting that manganese exposure exacerbates dopathiazine-mediated oxidative stress. Mn at micromolar concentrations accelerates the reoxidation of DTM1 and DTM2 reduced by dithiothreitol, H_2 or NADH and NADH-quinone oxidoreductase (NQO1). Other metal ions including Cu, Fe, Co, and Zn do not have this effect. The other dopathiazine, DTP, is more toxic to a dopaminergic cell line than DTM1 and DTM2, and its redox cycling is not stimulated by $MnCl_2$, indicating that it may independently play a role in dopaminergic degeneration in Parkinson's disease. We suggest that dopathiazines may occur naturally at low concentrations in the substantia nigra. Their deleterious actions may be elicited by complexing to Mn causing manganism or by an abnormal increase in concentration contributing to Parkinson's disease. Taurine, which scavenges hypochlorite, prevents the formation of dopathiazines and hence can be potentially therapeutic to both disorders.

Poster #29

Investigation of the Developmental Mechanisms of Hemimetabolous Scent Glands

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The presence of scent glands (ScG) is a characteristic trait found in many insect orders, ranging from Coleoptera (beetles) to Hemiptera (bugs). These specialized exocrine glands secrete a variety of volatile and odiferous compounds that serve to function chemical defense against predators as well as sex pheromones and alarm secretions. In the hemipteran *Oncopeltus fasciatus*, the scent glands are located on the fifth and sixth dorsal abdominal segments in nymphs and are translocated to the third thoracic segment in adults. Although these organs were studied anatomically and their secretions have been biochemically analyzed, very little is known about their ScG evolutionary origin and development. The dorsal scent glands consist of two parts; the secretory cell that manufactures the scent compounds and the duct cells that are positioned ventrally to the secretory cells which store the substances prior to excretion. The transcription factors *ventral vein-less* (*vvl*) and *trachealess* (*trh*) are expressed in the ScG primordia, with *vvl* in both the secretory and duct cells while *trh* is restricted to the duct cells only. Another transcription factor, *spalt* (*sal*), has been shown to localize only in the secretory cells during embryogenesis suggesting that *sal* is needed for the formation of the ScG opening in the cuticle. To begin to study scent glands development we performed *sal* maternal RNAi and confirmed its transcriptional depletion with *in situ* hybridization. Normally the wild-type shape of the scent glands resembles a "figure eight" shape and are equal in size in hatched 1st nymphs. Upon *sal* knockdowns the morphology of the scent glands became significantly altered, and the nymphs would die soon after emersion from the egg. We also observed a reduction of *vvl* expression in the secretory cell of the scent gland suggesting a genetic interaction of *sal* and *vvl* in that region of scent gland formation.

Recent analysis from our lab has revealed that *vvl* and *trh* are also necessary for the development of the tracheal system as well as the prothoracic and salivary glands in the head. Interestingly, these head glands are induced independently of *sal* whereas the development of the dorsal abdominal glands require an interaction between *vvl* and *sal* for their proper development. The results obtained so far suggest that the gland and tracheal systems originate from common primordia and are regulated by region specific genetic interactions, indicating that modulatory developmental mechanisms may lead to diversification of specialized structures in insects.

Poster #30

SinKM: A Signal Integration System Controlling the *Myxococcus xanthus* Developmental Program

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In response to its changing environment, most bacteria, including pathogens, control their behavior using Histidine-Aspartic Acid phosphorelay proteins, commonly termed "two-component signal transduction" (TCS) systems. A TCS consists of 1) histidine protein kinase (HPK) which contains a unique stimulus sensing domain fused to a histidine kinase domain, and 2) a response regulator (RR) which contains a phospho-accepting receiver domain fused to a DNA binding element. When a stimulus is sensed by the HPK sensor domain, the kinase is induced to autophosphorylate, and the phosphoryl group is then passed to the receiver domain of the RR which controls DNA binding to regulate gene expression. TCS systems have not been identified in mammalian cells, suggesting these signaling systems could be an important target to prevent bacterial diseases. In addition to the simple two-component system, some bacteria have evolved non-canonical HPK and identifying their role in signal transduction systems is challenging. These non-canonical HPKs are enriched in the bacterium *Myxococcus xanthus* and are involved in regulating its biofilm state. We are investigating how these non-canonical HPKs can regulate complex behavior using the histidine kinase, SinK, which consists solely of a histidine kinase domain followed by two receiver domains but lacks both sensing and output domains. Previous analyses have suggested SinK activity is controlled by a genus-specific hypothetical protein (SinM). We are currently working to understand how SinM modulates SinK activity. To determine if SinK and SinM directly interact, we are utilizing a bacterial two-hybrid (BACTH) system. Thus far, we have demonstrated that both SinK and SinM form dimers indicating both proteins are functional in this system. We do not detect interaction between SinM and the full-length SinK protein and are currently examining whether SinM interacts with specific domains of SinK.

Poster #31

Sculpting an imperfect flower: The study of *KNUCKLES-Like* in primordia regulation

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The evolution of sex determination in plants is a central problem in plant evolutionary biology. Currently, there have been limited studies in which the sex determination genes are identified yet we do not know most of the alternative downstream pathways that lead to developmental differences in plants that exhibit sexual dimorphism. Addressing this gap in knowledge is important as it will give insight into the genetic regulation of developmental processes in unisexual flowers and in angiosperm flowers in general. The investigation into the link between the differential expression patters of genetic pathways and the differential expression in floral development involves the differential expression of *AG, WUS*, and the proposed transcription repressor gene *KNUCKLES (KNU)* as they relate to the differential formation of floral organ primordia in male and female *Spinacia oleracea* flowers. Our central hypothesis is that the *AG-KNU-WUS* pathway regulates the differential morphogenesis of organ primordia between male and female flowers leading to sexual dimorphism in spinach. To test this hypothesis, molecular genetics tools are utilized to quantify *KNUCKLES* temporal and spatial expression patterns, along with functional testing. Preliminary studies have begun that include characterizing *KNU-like* gene expression and the phenotypes of *KNU-like* knockdowns in *S. oleracea*. **Preliminary results show specialization among three candidate genes. Phenotypic analyzes show an indeterminate phenotype in the axillary meristems and changes in whorl morphology and identity.**

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