Online Proceedings

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MOLECULAR AND CELLULAR BIOLOGY

Session Moderator: Hannele Ruohola-Baker, Biochemistry

Johnson Hall 175

1:00 PM to 2:30 PM

* Note: Titles in order of presentation.

Exome Sequencing Reveals De Novo Mutations in Schizophrenia

Amanda C (Amanda) Larson, Senior, Biology (Molecular, Cellular & Developmental)

Mary Gates Scholar

Mentor: Mary-Claire King, Medicine, Genome Sciences

Schizophrenia is a debilitating neuropsychiatric disorder with a worldwide prevalence of 1%. Although the heritability of schizophrenia is high, there is a large proportion of affected individuals with no family history. We are testing the hypothesis that many different severe mutations are the causal genetic factors for schizophrenia. In order to test our hypothesis, we sequenced the exomes of approximately 50 trios consisting of an affected individual and his/her parents who are free of mental illness. Exome sequencing is a rapid and robust method for looking at only coding portions of the genome, and allows for the detection of rare variants in those regions. Sequence data is then run through several bioinformatics filters to ensure that the variant is not common in the general population, and that the variant is probably damaging to the gene product. All variants that pass through the bioinformatics filters are validated with Sanger sequencing. De novo mutations were found in 19 different genes in 11 trios. Of the mutations 13 were single base pair changes, and 6 were truncating nonsense or insertion/deletion events. After these experiments have been carried out on approximately 100 trios, we will use the group of candidate genes to identify critical pathways that may be affected in schizophrenia. Understanding the structure of these pathways may allow for the development of targeted therapies for schizophrenia.

Biological Characterization of a Genomic Mutation Implicated in Schizophrenia

Molly Jeanette Gasperini, Senior, Biology (Molecular, Cellular & Developmental)

Levinson Emerging Scholar, Mary Gates Scholar, Undergraduate Research Conference Travel Awardee

Mentor: Mary-Claire King, Medicine, Genome Sciences

Mentor: Caitlin Rippey, Genome Sciences

Schizophrenia is a devastating neurodevelopmental disorder whose genetic influences continue to be elusive. Rare, gene-disrupting genomic deletions and duplications – called copy number variants (CNVs) – have been implicated in schizophrenia; however, much remains to be understood about which genes are causative, as well as the cellular mechanisms involved. Biological follow up of individual CNVs may give insight to the origin of schizophrenia. We focused on a CNV present in a patient with early onset schizophrenia. This mutation duplicates the 5' ends of two genes that lie head-to-head on chromosome 11q22: DCUN1D5, a previously uncharacterized gene predicted to be involved in cullin neddylation of ubiquitin ligase complexes, and DYNC2H1, a dynein active in cilia. Both are expressed in brain and are plausible candidate genes for schizophrenia. Using RNAseq, we detected novel DCUN1D5 transcripts in the patient's lymphoblasts that we predict will result in aberrant, truncated DCUN1D5 protein. To test the conformational stability of these aberrant proteins, we then expressed epitope-tagged full length and truncated DCUN1D5 in HEK293 cell lines. These transfected cells also allow us to study the functional capabilities of the aberrant proteins in the cullin neddylation pathway, and the specific cullin binding partners of DCUN1D5. Investigation of these aberrant proteins and their function may shed light on pathways that contribute to schizophrenia, potentially guiding the search for new candidate genes and the development of novel treatment strategies.

The Electrostatics of Ubiquitin and its Role in Ubiquitin Ligase Activity

Matthew Steven Novack, Senior, Biochemistry
Mentor: Rachel Klevit, Biochemistry
Mentor: Jonathan Pruneda, Biochemistry

Ubiquitin is a small post-translational signaling molecule. Its roles in repairing damaged DNA, apoptosis and protein degradation make it critical for tumor suppression. Genetic mutations affecting proteins in the ubiquitination pathway have been implicated in multiple inherited human cancers. The ubiquitination pathway attaches the protein ubiquitin (Ub) onto a substrate molecule with the help of three enzymes, ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin ligase (E3). Transfer of Ub onto the substrate requires the formation of a thioester bond between Ub and E2, denoted as E2. A catalytically active E3 ligase which can bind E2, but never directly binds Ub, is also necessary. E3's catalytic mechanism is not fully understood. One function of E3 is to bring E2and substrate in close enough proximity to facilitate a reaction. However, E3 enhances the release of Ub from E2in the absence of substrate molecules. This suggests E3 plays a role in priming E2for transfer of Ub. Using site directed mutagenesis and protein-protein interaction assays visualized through western blotting, I have investigated what electrostatic properties of Ub are important for E3's catalytic activity. Informative Ub mutations don't stop formation of E2, but disrupt the catalytic activity of E3. I propose that polar/charged Ub residues in the contact region between Ub and E2 affect E2binding modes, promoting inhabitance of E2conformations favorable to Ub transfer upon binding of E3.

Significance of Drosha Levels in MicroRNA Processing

Pratyusha Dutta (Trish) Banik, Senior, Biochemistry, Biology (Molecular, Cellular & Developmental)
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Henrik Sperber, Biochemistry

MicroRNAs (miRNAs) are small noncoding RNA molecules that post-transcriptionally regulate gene expression. MiRNA biogenesis involves two key ribonucleases, Drosha and Dicer. I am investigating the Drosha enzyme. Previous research from the Ruohola-Baker lab reveals that reducing levels of Drosha in stem cells significantly reduces levels of some miRNAs, while only slightly reducing levels of another group of miRNAs. This surprising result led us to conduct a bioinformatics analysis to identify characteristics that differentiate these two groups of miRNAs. We discovered structural differences, specifically that bulges in the central region of miRNAs were more frequent in heavily reduced miRNAs than in miRNAs that were not affected. My mentor, Henrik Sperber and I propose a model explaining why miRNAs with mismatches (bulges) in the central region of the sequence are more sensitive to reduced levels of Drosha than rigid miRNAs with no mismatches in that region. To test whether a bulge causes differential miRNA processing when Drosha is the limiting factor, I am adding and removing bulges to primary miRNA transcripts, overexpressing them in cells with low Drosha levels, and comparing their mature levels using qPCR. In addition, I am testing whether in nature cells vary Drosha levels as a mode of regulation. A previous study shows that T cells at specific stages of differentiation reduce Drosha expression by as much as 10 fold. My own bioinformatics analysis reveals that certain tissues in mice have an 8-fold reduction of Drosha. To see whether reduction of Drosha levels correlates with differential microRNA processing, I am using microarray data to find subsets of microRNAs that have dramatically reduced levels when Drosha expression is significantly low. With my research, I hope to discover a potentially novel mechanism of regulation in which cells alter Drosha levels to selectively process a subgroup of miRNAs.

***Pineapple Eye* Gene: A Novel Regulator of *Drosophila* Female Germ-Line Stem Cell Maintenance**

Jon Chu, Senior, Biochemistry, Biology (Molecular, Cellular & Developmental)
Mentor: Hannele Ruohola-Baker, Biochemistry
Mentor: Yalan Xing, biochemistry

Understanding stem cell maintenance is crucial for delineating conditions such as tissue loss, aging, and cancer. Using the *Drosophila* female germ-line stem cell (GSC) model to observe stem cells *in vivo*, several extrinsic and intrinsic factors have been demonstrated to regulate maintenance of *Drosophila* GSCs; they have also been found to be conserved in mammalian systems. The search is on for novel regulatory factors of stem cell maintenance with the hope of extending our understanding to stem cells in mammalian systems. My project involves the characterization of the molecular function of *Pineapple eye* gene (*Pie*), a novel regulator of GSC maintenance. Through a loss-of-function screen in GSCs, it has been demonstrated that mutations of *pie* result in GSC maintenance failure. The amino acid sequence of *pie* revealed that it contains ring-domains suggesting possible E3 ubiquitin ligase function. Such proteins are key components of the ubiquitination pathway, which are involved in protein tagging and degradation. To test *pie's* function as an E3 ligase, I am using a novel assay that employs the fact that bacteria do not have endogenous ubiquitination pathway and therefore utilizes *Escheria coli* as an *in vivo* system to express the components of the ubiquitination pathway and detect the formation of ubiquitin chains, a key indicator of E3 Ligase activity. I have demonstrated that the expression of *pie's* ring-domains without a substrate is not sufficient for the detection of ubiquitin chains. I will now test whether the full length *pie* is necessary for proper ubiquitination. Further, I will also test whether *pie's* functional domain needs a substrate in order to function effectively. Successful characterization of *pie* can

reveal new pathways to target for conditions involving stem cell maintenance.

Metabolism of Embryonic Stem Cells

Michael A (Michael) Choi, Senior, Biochemistry, Chemistry (ACS Certified)

Washington Research Foundation Fellow

Mentor: Hannele Ruohola-Baker, Biochemistry

Mentor: Wenyu Zhou

Embryonic stem cells are isolated from the early developing embryo and are capable of forming the different cell types found in the body. Understanding how these cells develop and maintain their specialized state is critical to understanding how they function. We hypothesize that embryonic stem cells acquire a unique metabolic state that aids them in maintaining their specialized state. Using two distinct mouse pluripotent states, embryonic stem cells (ESC's) and epiblast stem cells (EpiSC's) representing cells isolated from the inner cell mass of the blastocyst and the epiblast, a later stage in embryonic development, we found that EpiSC's are much more glycolytic in terms of their energy usage compared to ESC's as measured by oxygen consumption and extracellular acidification rates. Furthermore, using quantitative polymerase chain reaction experiments, we found that genes key to regulating and maintaining high rates of glycolysis are significantly upregulated in EpiSC's compared to ESC's. We showed that the overexpression of HIF1α, a known key regulator of these glycolytic genes, in ESC's is able to shift the ESC's towards a more glycolytic metabolic state and cellular morphology reminiscent of EpiSC's. In addition, transition of ESC's to EpiSC's using the known chemical inducers Activin and Fibroblast Growth Factor 2 revealed that changes in the expression of metabolic genes occur prior to changes in expression of cell fate markers, which suggest that a metabolic transition in the ESC's may be key in its transition to an EpiSC-like state.

Induced Pluripotent Stem Cells: The Impact of Histone Methylation on Reprogramming

Akiko Alison (Akiko) Carver, Junior, Biology (Molecular, Cellular & Developmental)

Mentor: David Hawkins, Medicine & Genome Sciences, University of Washington School of Medicine

Mentor: Jo Ling Liao, Medical Genetics

Human embryonic stem cells (hESCs) are pluripotent, or able to differentiate into all cell types of a developing embryo. They can be used to engineer human tissue, but are prone to immune rejection when used in medical treatment. Similar to hESCs but derived from the reprogramming of adult somatic cells, human induced pluripotent stem cells (hiPSCs) were developed that have the potential for use in regenerative therapy with less risk of rejection. However, hiPSC research faces its own challenges, such as inefficient reprogramming rates and variation in observed pluripotency. Previous research has shown that methylation patterns of hESCs can be distinguished from hiPSCs at both the DNA and histone level, suggesting less pluripotency in hiPSCs than in hESCs. While exhibiting greater overall methylation on DNA but less on histones, many of the hypermethylated DNA regions of hESCs are adjacent to these differentially methylated histones. The location of the hypermethylated regions suggests that erasing specific histone methylation marks may improve hiPSC reprogramming rates by creating a more open chromatin landscape, and may create a cascade effect that allows for additional methylation of the genome. This experiment investigates the correlation between histone methylation and hiPSC reprogramming efficiency and accuracy. We reprogrammed human fibroblast cells with lentiviruses expressing various demethylases and transcription factors in order to compare resultant hiPSC methylomes to traditional hiPSC and hESC lines. Opening of the chromatin is expected to improve reprogramming rates and allow for proper methylation of the genome, creating an hiPSC closer in phenotype to hESCs and more useful for clinical therapies.