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Effects of Aneuploidy in *Saccharomyces cerevisiae*

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Aneuploidy has long been studied in regards to its relationship with cancer and many other diseases, but the specific cellular processes that are altered by aneuploidy are unknown. Through analysis of disomic strains of yeast, strains that contain an extra copy of one chromosome, we hope to characterize the effects of aneuploidy. Analysis of RNA and protein content of disome II reveals a number of genes on chromosome 2 that show a twofold increase in RNA levels, but not in protein levels. These proteins are mostly involved in complexes, and may be degraded in order to maintain stoichiometric protein concentrations. To gain a better understanding of the specific effects of aneuploidy, the disomic strains were evolved, and sequenced to identify mutations that may increase their tolerance of aneuploidy. One of the mutations that was isolated was in the gene *ubp6*, which encodes a deubiquitinase that has been shown to delay degradation of some proteins. Competition assays were used to analyze disomic strains with a deletion in *ubp6* and it was found that the deletion increases the fitness of the disomes V, VIII, and XI in selective media to a greater extent than the wild type. We propose that protein degradation plays an important role in maintaining protein balance in the disomic strains, and the deletion of *ubp6* may play a role in upregulating protein degradation in certain aneuploid strains.

Faculty Supervisor: Angelika Amon
Postdoc Mentor: Eduardo Torres

SirT1 Regulates Bone Remodeling *in vivo*

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Bone remodeling is regulated by the coupled action of two different cell types: the mesenchymal-derived osteoblasts that form bone and the hematopoietic-derived osteoclasts that break down bone. Osteoporosis occurs when this process becomes uncoupled, either through downregulation of osteoblast activity, upregulation of osteoclast activity, or a combination of both (Harada and Rodan, 2003). SirT1, the mammalian homologue of yeast Sir2, is an NAD+ dependent histone deacetylase implicated in genomic silencing and aging (Blander and Guarente, 2004). SirT1 has recently been shown to be a repressor of PPARγ, the master fat transcription factor. As both osteoblasts and adipocytes are derived from mesenchymal stem cells, and differentiation down one pathway precludes differentiation down the other, it is possible that SirT1 is also a regulator of osteoblastogenesis. Moreover, SirT1 has also been shown to repress NFκB, a major osteoclast transcription factor. Consistent with the above premise, SirT1 knockout mice have dramatic bone deficiencies, including a decrease in bone mineral density and osteoblast numbers, as well as an increase in osteoclast numbers. SirT1 may therefore regulate bone remodeling by promoting osteoblast and inhibiting osteoclast differentiation. To confirm this hypothesis, we created osteoblast and osteoclast-specific SirT1 knockout mice and showed that both groups of mice also display early onset osteoporosis. Further, osteoblast-specific knockouts had a lower number of osteoblasts, while osteoclast-specific knockouts had a higher number of osteoclasts, confirming SirT1's cell autonomous role in the differentiation of each cell type. Currently, we are determining the mechanism by which SirT1 exerts its effects in these two principle bone cell types.

Faculty Supervisor: Leonard Guarente
Graduate Mentor: Kayvan Zainabadi

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Sensitizing Cancer Cells to Cisplatin by Targeting Translesional Polymerases Using RNAi

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Cisplatin, a platinum-based chemotherapeutic agent that forms DNA adducts, is commonly used to treat many different types of cancer. Some cancers can become resistant to cisplatin, however, but the genes involved in this chemotherapeutic resistance remain largely unknown. We used RNA interference (RNAi) to study the role of Rev3 in cisplatin resistance. Rev3 is the catalytic subunit of DNA Polymerase Zeta, an error-prone translesional polymerase that is able to replicate past various DNA lesions. We found that knocking down Rev3 using short hairpin RNAs (shRNAs) in lymphoma cells greatly sensitizes the cells to cisplatin both *in vitro* and in an *in vivo* mouse model. We have also started looking at the effects of Rev3 knockdown in several lung cancer lines, and have found that these cells are sensitized to cisplatin as well. We are currently looking at the effects of Rev3 knockdown in lung cancer *in vivo*. Our findings show that the translesional polymerase Rev3 is important in mediating cisplatin resistance and suggest translesional polymerases as possible drug targets to sensitize tumors to DNA-damaging chemotherapeutics such as cisplatin.

Faculty Mentor: Michael Hemann
Graduate Student Mentor: Jason Doles

The role of genetic variation in the expression of the MHC and susceptibility to multiple sclerosis

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Various alleles within the major histocompatability complex (MHC), such as HLA-DRB1*1501, have been identified as risk factors for autoimmune diseases, but it is not clear how these variants exert an effect on risk of disease. We genotyped SNPs in 4.4 Mb of the MHC in 270 human lymphoblastic cell lines derived from HapMap individuals. We identified seven loci that are significantly associated with gene expression. We tested these SNPs for association with susceptibility to multiple sclerosis (MS), but none of them had a significant effect after the effect of HLA-DRB1*1501 was taken into account. Differences in gene expression may still impact disease risk, for HLA-DRB1*1501 was nearly always inherited with the major alleles of SNPs that have an effect on HLA-DRB1 and HLA-DQA1 expression. Thus, SNPs associated with expression of MHC genes have no independent effect on susceptibility to MS, but they may modulate the risk associated with the established susceptibility allele HLA-DRB1*1501.

Faculty Supervisor: David Hafler

Let-7 MicroRNA Regulation of Non-Small Cell Lung Cancer

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MicroRNAs are short, endogenous, non-coding RNAs that regulate the expression of target mRNAs and are often found aberrant in human cancers. The let-7 family of microRNAs has been proposed to function as a key tumor suppressor in human non-small cell lung cancer (NSCLC). Let-7 levels are often found down-regulated in lung cancer patients; in addition, let-7 has been shown to negatively regulate the expression of several oncogenes, such as c-Myc, HMGA2 and the Ras family, and repress the proliferation of human cancer cell lines *in vitro*. Recent work has demonstrated that let-7 is able to suppress NSCLC initiation in an autochthonous mouse model of cancer. To better understand how let-7 mediates tumor suppression in NSCLC, using a genetically engineered mouse model, we further characterized the expression of known let-7 targets (K-Ras, HMGA2, c-Myc) in response to let-7 induction in naturally arising lung tumors. Upon let-7 induction, previously characterized let-7 targets had robustly reduced expression, demonstrating that let-7 is expressed and functional in these tumors. We then further characterized the activation of several targets downstream of Ras (Akt, GSK-3, TSC2, Mek and Erk) in response to let-7 induction. In let-7 expressing tumors, the levels of phosphorylated Akt, GSK-3, TSC2, and Mek generally decreased over longer periods of let-7 induction; however, these levels varied within a population of mice with the same let-7 treatment. In contrast, the phosphorylated levels of Erk remained mostly constant over sustained let-7 induction. Finally, we strived to determine the contribution of activated intermediates, MEK-^{DD}, Akt1-^{myr} and RalA^{G23V}, in the MAPK, PI3K, and RalGDS pathways respectively downstream from Ras, as well as the cell-cycle regulators CDK6, CyclinD2 and CDC25A, in the rescue of let-7 mediated tumor suppression.

Faculty Supervisor: Tyler Jacks
Graduate Student Mentor: Madhu S. Kumar

Production of 2,3-Butanediol in *Escherichia coli*

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The field of metabolic engineering has provided the tools and techniques necessary to create microorganisms capable of producing a wide variety of natural and non-natural compounds, essentially turning these microorganisms into miniature chemical factories. This project uses these techniques to reconstruct the (*R,R*)-2,3-butanediol pathway in *E. coli* to create a possible platform for industrial-scale production of this chiral diol, which has many current and potential commercial applications. Initial experiments and analysis showed that *E. coli* BL21Star(DE3) strains grown at 30°C, under anaerobic conditions and with rich media supplemented with 1% (w/v) glucose achieved the highest titers of 2,3-butanediol. Host engineering was done to further maximize production levels. Results show that with a lactate dehydrogenase deletion, BL21Star(DE3) strains achieved significantly higher maximum titers than the wild-type. In particular, those strains expressing the pathway comprised by the *ilvBN,aldC,butB* gene combination attained a maximum titer of 1 g/L 2,3-butanediol.

Faculty Supervisor: Kristala Jones Prather
Postdoc Mentor: David R. Nielsen

End_Labeling and Cyclization of Proteins Using Sortase A

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Sortases are membrane-bound enzymes found in gram-positive bacteria that recognize conserved motifs on proteins and "sort" these proteins by anchoring them to the cell wall through a transpeptidation reaction. In particular, Sortase A (SrtA) from Staphylococcus aureus recognizes the LPXTG motif, cleaving it between the threonine and glycine residues (forming a protein-LPET-SrtA intermediate) and ultimately transfering it to the free N-terminus of a glycine nucleophile. This transpeptidation reaction proceeds smoothly *in vitro* and has emerged as a new tool for protein engineering.

We have made use of recombinant SrtA to site-specifically label both termini of proteins *in vitro* with custom-made small molecules. By installing an LPXTG motif onto the C-terminus of a protein, we can use SrtA to append various functional groups such as fluorescent dyes, photo-crosslinkers, or lipid tails. Recently, we have developed a complementary method that uses synthetic mimetics of the LPXTG peptide to install modifications on the protein N-terminus, provided the protein contains an accessible N-terminal glycine. We are exploiting the ability to label both termini to develop a general strategy for dual labeling of the same polypeptide. Progress toward this goal involves a unique masking strategy whereby the protein N-terminus is blocked by a protease cleavage site and revealed after successful labeling of the C-terminus.

The potential for transpeptidation reactions at both N and C termini has further led to the development of a unique cyclization reaction, whereby the N-terminal glycine is ligated to the C-terminus of proteins containing a LPXTG motif. Circular proteins are known to shown improved resistance to denaturation and proteolysis, and the intramolecular transpeptidation approach mediated by SrtA may provide a simple means for accessing circular proteins of therapeutic value.

Faculty Supervisor: Hidde Ploegh
Postdoc Mentor: John Antos

Functional Consequences of Oligomerization in CPG15

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The complex wiring of the brain gives an organism the ability to interact with and adapt to the surrounding environment. In cases of neurodegenerative disease or injury, dendritic arbor retraction and neuronal death dramatically disrupt brain circuitry leading to debilitating losses of motor skills, memory and cognitive function. It is thus of critical importance to identify molecules that promote cell survival and neurite outgrowth. We have identified a molecule, candidate plasticity gene 15 (CPG15), that promotes both outgrowth and survival of neurons. However, its low expression levels ***in vitro*** and presumptive aggregative properties have limited our understanding of its function and potential therapeutic potential. Here we report the generation of a variant of CPG15, referred to as tC75S, in which the glycosylphosphatidylinositol (GPI) anchor and the cysteine at position 75 have both been removed. This variant expressed at ~10ng/µl (roughly 5 times higher than wildtype levels) and, unlike tCPG15 with all cysteines present, retained wildtype functions including activation of Akt and Erk phosphorylation and promotion of neurite outgrowth. Further characterization of CPG15 oligomerization revealed that both the GPI linkage and cysteines 75 and 111 were not essential for activity. To elucidate the mechanism of CPG15 in neuronal survival and outgrowth, we plan to use the optimized CPG15 variant to identify the cellular receptor of CPG15 and effectors of its activity.

Faculty Supervisor: Elly Nedivi
Postdoc Mentor: Alan Marnett

miR-9, an E-cadherin-suppressing microRNA, induces vascular endothelial growth factor and cancer metastasis

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MicroRNAs (miRNAs) are small non-coding RNA molecules that suppress gene expression by interacting with multiple target mRNAs. These molecules are just beginning to be implicated in the malignant progression of cancer. In an initial qPCR-based screen for miRNAs differentially expressed in human breast cancers, our lab identified three miRNAs that are most significantly upregulated in human breast cancer cells. The subsequent functional studies of miR-10b validated its candidacy as a metastasis promoter. Working with Dr. Li Ma, I find that miR-9, which stood out in the previous screening as a second miRNA upregulated in breast cancer cells, directly targets the E-cadherin-encoding mRNA, *CDH1*, leading to a cell type- and context-dependent epithelial-mesenchymal transition (EMT) and increased cell motility and invasiveness. Furthermore, miR-9-mediated E-cadherin downregulation results in the activation of the β-catenin signaling, which in turn upregulates the β-catenin target, vascular endothelial growth factor (VEGF), leading to tumor angiogenesis. Overexpression of miR-9 in otherwise non-metastatic breast tumors induces formation of pulmonary micrometastases in mice. Conversely, inhibiting miR-9 using the 'miRNA sponge' in highly malignant cells suppresses metastasis formation. Interestingly, expression of miR-9 might be activated by multiple mechanisms in tumor cells, including demethylation, Ras and possibly EMT-inducing transcription factors. These findings represent the identification and characterization of a unique metastasis-promoting miRNA, miR-9, which is the only miRNA that is predicted (and validated here) to target the metastasis suppressor, E-cadherin.

Faculty Supervisor: Dr. Robert A. Weinberg
Postdoc Mentor: Dr. Li Ma

The role of RNA polymerase II pausing in divergent transcription

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Recent evidence suggests that many genes in the human genome exhibit divergent transcription. Divergent transcription is the process by which two distinct RNA polymerase II (RNAPII) complexes form at a transcription start site and initiate in opposite directions. Polymerase pausing is an event that has been previously characterized and involves many complexes including DSIF and NELF. Divergent transcription is hypothesized to be partially regulated by promoter proximal pausing of RNAPII. The proposed model suggests that the polymerases pause after initiation at which point they can be further regulated to transition into a productive elongation phase. One characteristic of divergent transcription is the generation of transcription start site-associated RNAs (TSSa-RNAs) which surround active promoters in both the sense and antisense orientations. To test for the importance of the two pausing complexes, DSIF and NELF, in TSSa-RNA formation we used siRNA technology to deplete specific components of these complexes and northern blot analysis to visualize the resulting TSSa-RNA population. The DSIF complex has been shown to regulate both polymerase pausing and elongation while the NELF complex is only involved in pausing. Here, we present evidence that spt5, a subunit of DSIF, impacts the generation of TSSa-RNAs and by extension, divergent transcription. Northern analysis shows a shift to shorter TSSa-RNA transcripts when spt5 is depleted. Changes to the TSSa-RNA population are not observed when NELF-A, a component of the NELF complex is silenced. Depletion of the spt5 subunit of DSIF also leads to a change in the distribution of RNAPII in transcribed genes, as visualized through chromatin immunoprecipitation followed by high-throughput sequencing. These results suggest that the transition of a polymerase from a paused state to an elongating state may be critical for TSSa-RNA biogenesis.

Faculty Supervisor: Phillip Sharp
Postdoc Mentor: Amy Seila

Tissue-specific TIR-1/NSY-1/SEK-1-dependent MAPK activation confers pathogen resistance through neuroendocrine signaling in *C. elegans*

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A conserved TIR-1/NSY-1/SEK-1/PMK-1 MAPK pathway is required for immunity in *C. elegans*. We investigated the tissue-specific functions of TIR-1/NSY-1/SEK-1-dependent MAPK signaling in response to pathogen infection. We determined that TIR-1/NSY-1/SEK-1-dependent MAPK signaling in the intestine provides a major contribution to pathogen resistance, but does not completely restore pathogen resistance. Surprisingly, we defined an additional tissue-specific role for TIR-1/NSY-1/SEK-1-dependent MAPK signaling in chemosensory neurons in resistance to pathogen killing. We are currently defining the neuroendocrine signaling circuitry that functions downstream of TIR-1/NSY-1/SEK-1-dependent MAPK signaling to confer pathogen resistance in *C. elegans*.

Faculty Supervisor: Dennis Kim

The *Toxoplasma gondii* Protein JLP2 Modulates the Host NFKB Pathway

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Toxoplasma gondii is an obligate, intracellular, brain parasite that infects approximately a third of the world population. This protozoan also has a wide host range making *T. gondii* easy to culture and an important model parasite. We studied the strain specific differences between the three most common strains of *T. gondii*, Types I, II, and III with respect to activation of the host cell NFkB pathway during infection. Activation of the NFkB pathway leads to an immune response by the host. Previous studies suggest that one of the proteins injected by *T. gondii* upon invasion interacts with and initiates the NFkB pathway. This was determined by Immunofluoresence Assay (IFA) antibody tagging of p65 which travels to the nucleus from the cytoplasm as part of NFkB activation. This translocation is necessary in order to activate NFkB, a transcription factor that transcribes genes for pro-inflammatory cytokines. However, this only happens with Type II *T. gondii* infections and not Type I or III. We preformed fine mapping with Type II x Type III F1 progeny to localize the responsible gene in the Type II strain to a section on *T. gondii* chromosome X. Specific candidate criteria narrowed the candidate genes to 6 possibilities, one of which was JLP2. I tested JLP2 by inserting a Type II version into a plasmid that was then transformed into a Type I *T. gondii*. Analysis by IFA of the recombinant Type I *T. gondii* with Type II JLP2, showed that the Type I strain, previously negative for NFkB activation, became positive with the presence of the Type II JLP2 protein. This indicates that JLP2 interacts with the host NFkB pathway. Future work includes identification of the host cell protein that interacts with JLP2 by immunoprecipitation and crystallization of the protein for mechanism analysis.

Faculty Supervisor: Jeroen Saeij