Genetics Day Poster Submission

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**Title:** THE MECHANISM OF HISTONE EXCHANGE BETWEEN LIPID DROPLETS

**Abstract:** Lipid droplets (LDs) are highly conserved organelles that have recently been shown to play active roles in fundamental regulatory processes such as protein sequestration, with the best studied example being histone proteins H2A, H2Av, and H2B in Drosophila melanogaster. The variant histone H2Av exchanges (transfers back and forth) between LDs in early Drosophila embryos via transient sequestration to the LD protein Jabba. This exchange mechanism is dynamic and paces the nuclear import of H2Av, which is critical for downstream regulation of chromatin assembly and gene expression. Exchange of H2Av is dynamic for a bit over 2 hours but then suddenly (over less than 15 min) stops and the transient Jabba-H2Av interactions become static. This switch in H2Av behavior occurs during the midblastula transition (MBT), a critical developmental time for the embryo when zygotic transcription is massively upregulated, nuclear divisions pause, and cellularization is initiated. Prior to the MBT, the nuclei undergo rapid, synchronous divisions without cytokinesis to quickly increase nuclear content in the embryo. The ratio of nuclei to cytoplasm (N:C ratio) is one of the factors that determines when MBT events happen. The mechanism driving the switch in H2Av dynamics and its biological function are unknown. Therefore, we aim to answer the central question: How is the exchange of H2Av between LDs temporally regulated? I will present preliminary on two candidate molecular players that could be regulating H2Av dynamics: Importin alpha 2 (Imp$\alpha$2) and Cyclin-dependent kinase 1 (Cdk1). Imp$\alpha$2 was identified as a candidate regulator via proteomics, interacts directly with Jabba, and is necessary for H2Av exchange between LDs. As H2Av exchange halts at the MBT, Imp$\alpha$2 both transitions to an unphosphorylated state and becomes enriched on LDs, leading to the hypothesis that the phosphorylation status of Imp$\alpha$2 controls H2Av dynamics. Cdk1 activity is massively downregulated at the MBT; this downregulation has been proposed to be critical for how the N:C ratio controls the timing of MBT events. Because the switch in H2Av dynamics is regulated by the N:C ratio, Cdk1 is an attractive candidate for controlling the switch. Furthermore, Cdk1 phosphorylates the mammalian ortholog of Imp$\alpha$2, and there is a predicted Cdk1 site on Imp$\alpha$2. I hypothesize that this kinase acts by controlling Imp$\alpha$2 phosphorylation, which in turn regulates H2Av dynamics. Therefore, I propose to test Imp$\alpha$2 and Cdk1 as regulators of H2Av exchange. More specifically, I will generate phosphomimic and unphosphorylatable mutants of Imp$\alpha$2 and disrupt Cdk1 activity with inhibitors and cyclin knockdown. If Imp$\alpha$2 phosphorylation is important for H2Av exchange, I will then probe if it is affected by changes in Cdk1.