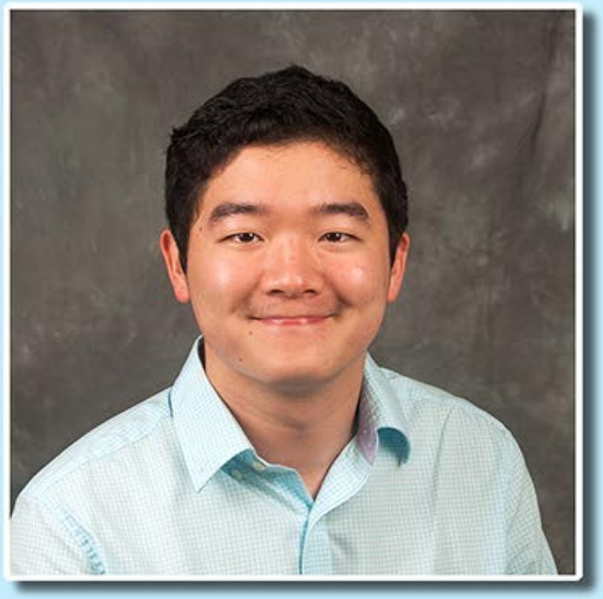

Samuel Chao

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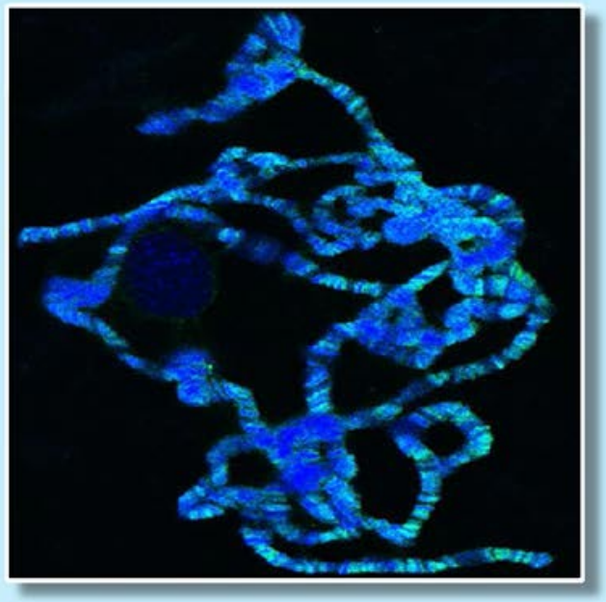

Polytene Chromosome In S-Phase
(blue: DAPI, green: anti-PCNA, red: anti-HP1)

Investigating Histone Regulation of Metazoan Replication Timing In Vivo

Accurate DNA replication during cell proliferation is necessary for proper human development as well as the avoidance of many diseases. Replication is an asynchronous event, as many sites across the genome carry out initiation at various times throughout S-phase of the cell cycle, a process called the replication-timing (RT) program. Aberrations in RT have been associated with cancer, yet the exact mechanisms underlying the control of RT remain largely unknown. We hypothesize that the modulation of chromatin structure by histone post-translational modifications (PTMs) plays an important role in the regulation of RT. Heterochromatic PTMs (i.e. H3K9me) are associated with late S-phase replication while euchromatic PTMs (i.e. H4K16ac) are associated with early replication. A previously-developed histone gene replacement platform in *Drosophila* was employed to mutate specific histone residues (e.g. H3K9R and H4K16R) and to prevent certain PTMs. First, the effects of histone mutation were assessed in diploid cells by using flow cytometry to observe cell cycle progression from G1 to S phase. H3K9R mutants had an abnormal proportion of cells in G and S-phase whereas H4K16R mutants did not, demonstrating that the PTM of H3K9 is necessary for proper cell cycle progression in *Drosophila*. Second, to more closely examine S-phase progression itself, the effects of histone mutation were assessed in polyploid cells, using immunofluorescence to detect when various regions of the genome replicated. H4K16R mutants performed abnormally synchronous replication of both euchromatic and heterochromatic regions, suggesting that this PTM plays a role in regulating the RT program. Since abnormal RT is error-prone, RT dysregulation may contribute to the emergence of cancer. Therefore, exploring the interrelationships among histone PTMs, chromatin structure, and regulation of RT may provide a greater understanding of the underlying causes of cancer.