

Studying the Role of Post-translational Histone Modifications in Gene Regulation

Sarah Anderson
BS Biology/BA Chemistry

Dr. Bob Duronio
Dept. of Biology

With special thanks to:
Pam Malek
Lab Manager



Background and Goals

- Histones are packaging proteins that help organize DNA into chromatin. They also contribute to gene regulation.
- Histones are hypothesized to contribute to gene regulation by undergoing chemical modifications known as post-translational modifications (PTMs).
 - However, this has never been tested directly.
- The Duronio Lab plans to use the fruit fly *Drosophila melanogaster* to test this hypothesis by genetically engineering flies with mutated histone genes that cannot be chemically modified. Flies are the only commonly used experimental organism in which this is possible.

- Goal: To create lines of flies with transgenic histone genes containing mutations inhibiting certain histone PTMs in order to determine their function.
 - Objective 1: To create histone DNA that can be used to make a transgenic fly carrying multiple wild type histone genes.
 - Objective 2: To create mutations in the histone gene cluster that will prevent certain PTMs and to create transgenic flies carrying these mutations.

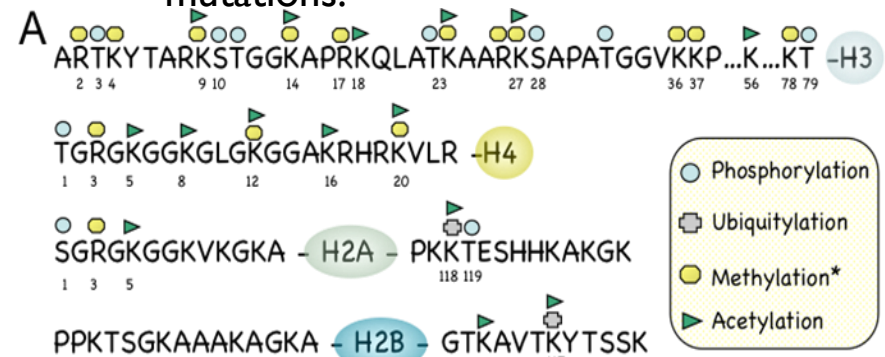
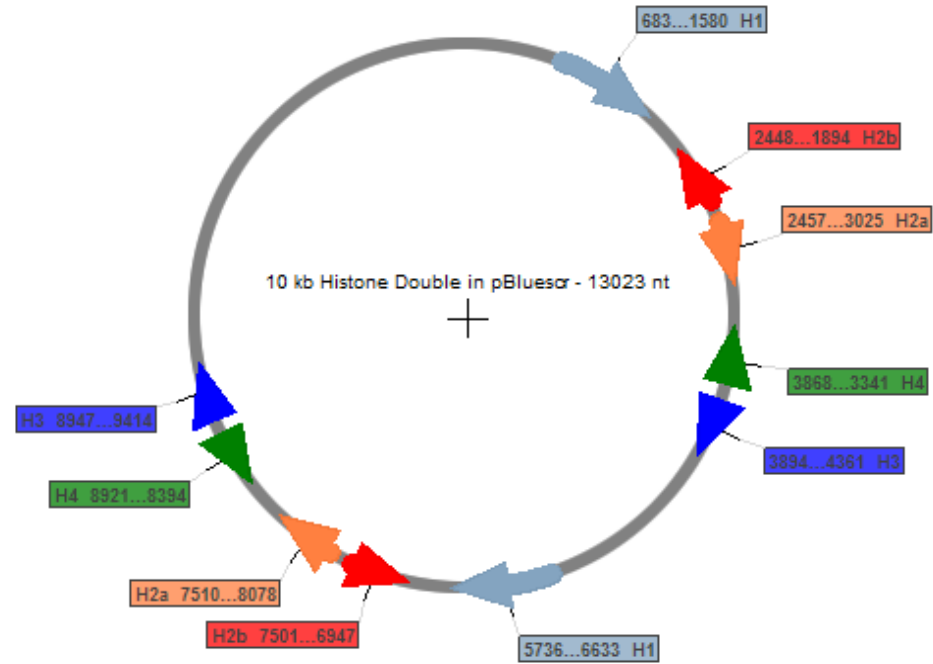


Diagram of where the different types of histone PTMs occur.

Results

- Objective 1:
 - We want to create a plasmid containing 12 copies of the histone gene.
 - I was able to clone plasmids containing 2 repeats of the histone gene cluster.
 - Future Goals: To use a Bac plasmid backbone to create 12 copy repeats of the histone cluster, which can be used to make transgenic flies.
- Objective 2:
 - I was able to create 8 different mutations that change lysines (can be chemically modified) to alanines (cannot be modified).
 - Future Goals: To create 12 copy repeats of these mutated histone gene clusters, and to make transgenic flies.



Map of pBluescript plasmid containing 2 repeats of the histone gene cluster (objective 1).

histone	modification	abbreviation	biological function	readers	writers
H3 lysine 4	trimethylation	H3K4me3	gene activation	NURF/CHD1 ATPases;	Set1/Ash2/trithorax
H3 lysine 9	trimethylation	H3K9me3	heterochromatin formation	HP1	Su(Var)3-9/G9a
H3 lysine 14	acetylation	H3K14ac	gene activation	RSC ATPase	CBP/p300/Gcn5
H3 lysine 27	trimethylation	H3K27me3	developmental gene silencing	Polycomb (Pc)	E(Z)
H3 lysine 36	di- & trimethylation	H3K36me2/me3	transcriptional elongation	Rpd3S deacetylase	Set2/Mes-4
H4 lysine 16	acetylation	H4K16ac	gene activation/dosage	TFIID/Brd4	MOF
H4 lysine 20	methylation	H4K20me1/me2	silencing/cell cycle regulation	p53BP1	PR-Set7/Su(var)4-20/dSET8

7 of the 8 mutations that I created this summer (objective 2).