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DISSERTATION

"EXPLORING THE ROLE OF CHROMATIN DECONDENSATION AT A GENE LOCUS IN STOCHASTIC GENE EXPRESSION"

by

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Molecular Biology, Genetics, & Cancer Track

B.S., 2011, University of New Haven, West Haven, CT
M.S., 2013, University of Connecticut, Storrs, CT

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Friday, April 12th, 2019
12:00 P.M.
ICPH Auditorium

ABSTRACT

Individual cells within isogenic populations show great variability or "noise" in gene expression. This variability is exploited by unicellular organisms for adaptation. Cancer relapse is suggested to be a consequence of variable gene expression, because it allows small fractions of cells to escape cancer therapy. A previous model suggests that noise is due to the random opening and closing of chromatin at the promoters, creating "on" and "off" states of gene expression. We aim to characterize the molecular mechanisms involved in the noisy gene expression by perturbing the chromatin specifically at a single gene locus.

We constitutively tethered the catalytic histone acetyltransferase domain of p300, a transcriptional co-activator, to the regulatory regions of the cyclooxygenase-2 (Cox-2) and collagenase I (Col I) genes. This constitutive tethering was accomplished by fusing the histone acetyltransferase domain of p300 to an endonuclease-deficient CRISPR-associated protein 9 (dCas9). Stable cell lines were created that express the dCas9 system along with small guide RNAs designed to guide the fusion protein to the target genes. We show that tethering a histone acetyltransferase to regulatory regions of the target genes cause a significant increase in chromatin accessibility rendering the locus constitutively open.

We then measured the cell-to-cell variability in the expression of the Cox-2 and Col I genes in cell lines expressing the dCas9 system compared to the variability of the same genes in unmodified cells. To measure the variation in gene expression, we imaged and quantified each target mRNA in single cells using single-molecule FISH and then statically characterized the cell populations. Our results show that cells with the Cox-2 or Col I promoter region that are constitutively open due to tethering of histone acetyltransferase at their promoters, exhibit reduced variability in the number of mRNA transcripts. These results support the hypothesis that promoter accessibility plays an important role in heterogeneous gene expression.

Through an understanding of the fundamental molecular mechanisms of gene expression, future studies can be performed to control or lessen the magnitude of gene expression variability within cell populations, enabling an exploration of the phenotypic consequences.