Chromatin architecture and the orchestration of gene expression: cell systems to explore epigenetic gene control

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Summary

Chapter 1 is an introductory chapter, presenting the current view of eukaryotic gene control with respect to the spatial organization of the genome within the cell nucleus. Eukaryotic gene regulation is achieved at several levels. For instance, at the genetic level or linear DNA-sequence level, genes with a similar activity level can occur in clusters, suggesting that the genomic environment can contribute to transcriptional activity. Furthermore, at the chromatin level, active genes often seem to loop away from their chromosome territory, demonstrating a correlation between chromatin organization and transcription. Finally, the radial organization of the genome within the nucleus likely exerts regulatory influences. A more peripheral position can have a silencing effect on genes, as opposed to a more central nuclear position.

In **chapter 2** we utilized Chinese Hamster Ovary cells containing an amplified lac operator array to examine how MeCP2, a protein embedded in the DNA methylation pathway, influences epigenetic regulation. We demonstrated that the *in vivo* targeting of MeCP2 caused extensive chromatin decondensation requiring the C-terminal part of MeCP2. MeCP2-induced chromatin decondensation occurred throughout interphase in the absence of transcriptional activation or changes in CpG methylation. We demonstrate an intricate interplay between MeCP2 and HP1 proteins by showing that MeCP2 promotes eviction of HP1γ from chromatin, but not of HP1α or HP1β. We propose that MeCP2-induced chromatin decondensation reflects a poised status, preparing chromatin for further transcriptional regulation.

In **chapter 3** we studied whether HP1 that lacks a functional chromodomain (CD), normally mediating binding to chromatin via histone H3 tri-methylated at lysine 9, is able to induce heterochromatinization. After targeting of CD-less HP1 we observe chromatin compaction, locally enhanced H3K9me3 levels and recruitment of endogenous HP1α and HP1β to the targeted region. These results suggest that recruitment of factors by the chromoshadow and hinge domain of HP1 is sufficient to induce heterochromatinization.

In **chapter 4** we present the effects on chromatin structure of the depletion of endogenous HP1 from mouse chromocenters. We used a dominant-negative approach by expressing truncated HP1 lacking a functional CD in mouse fibroblasts, resulting in a reduction of the accumulation of endogenous HP1 in pericentromeric heterochromatin domains. The expression levels of HP1 did not change. The displacement of HP1 from pericentromeric heterochromatin domains did not result in visible structural changes as visualized by DAPI staining and H3K9me3 labeling. Our data indicate that accumulation of HP1 at pericentromeric heterochromatin domains is not required to maintain such domains.

In **chapter 5** we describe the creation of an engineered targeting system in human cells and discuss its benefits and points for improvement. The distinguishing feature of this targeting system is its single-copy integration into two cell lines in a predefined well-characterized genomic location exhibiting opposing characteristics. This system enables us to study the effect of the endogenous genomic environment on gene expression and chromatin structure. Furthermore, two small targeting sites incorporated in the engineered construct can be targeted simultaneously by different epigenetic factors that will provide information on factor interactions and cross-talk. These targeting sites have been limited in size to reduce potential artifacts that can arise from the integration of exogenous DNA. Pilot studies demonstrate the potential of our novel targeting system.

Chapter 6 is a perspective in which we discuss eukaryotic gene network models and their use in gaining insight into the gene regulatory system. We offer insight into the role of epigenetics in biological systems, including a proposal for the integration of the epigenetic layer in gene network models.