# Genomic mapping of heterochromatin components using DamID

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## Thesis Outline

Chromatin is the complex of DNA, RNA and protein that makes up chromosomes. Chromatin regulates many important processes in the nucleus, including transcription, DNA replication and repair. Heterochromatin is a chromatin subtype, which can be defined molecularly by its components HP1 and H3K9 methylation.

We have optimized a new powerful mapping technology named DamID, which enabled us to identify the genomic binding sites of heterochromatin proteins. We have used bioinformatics approaches to integrate our binding profiles with various genome-wide data sets. The research presented in this thesis describes the construction and analysis of these genome-wide heterochromatin binding profiles.

**Chapter 1** is the general introduction and discussion. We summarize and discuss past and recent observations linking heterochromatin to gene regulation. Heterochromatin is found on both active and repressed genes, but how heterochromatin functions in these two contexts was not clear. Based on our own results and studies by others, we propose a model that reconciles the silencing and activating qualities of heterochromatin. This model is discussed in relation to our own findings that are presented in chapter 3 and 4.

The DamID method was originally developed in a *Drosophila* cell line. We adapted DamID for use in human and mouse cell lines, which was not trivial because of the mammalian specific adjustments that had to be made. The protocol for mammalian DamID together with discussion of its principles, advantages and limitations is presented in **chapter 2**.

In order to understand the function of heterochromatin in the mammalian genome it is essential to know the DNA sequences that are covered in heterochromatin. In **chapter 3**, we present the first genome-wide binding maps of heterochromatin proteins in mammalian cells. Unexpectedly we found that most members of the KRAB-ZNF gene family are bound by heterochromatin proteins. We speculate that heterochromatin has a role in the evolution of this gene family.

In **chapter 4** we focus on the longstanding observation that euchromatic genes become silenced when they are moved to heterochromatic regions of the genome, a phenomenon known as PEV. To obtain insight into the mechanism of silencing, we mapped HP1 binding in a *Drosophila* PEV-model. To do this we optimized the DamID method, which enabled us to work with very small tissue samples (dissected fly heads). We find that in the PEV model, HP1 invades a ~200 kb region of euchromatin, causing *de novo* association of HP1 with 20 genes. HP1 binding levels in this ~200 kb region show substantial local variation, which might explain why only 2 genes are substantially repressed by HP1.

In summary, technical improvements of DamID allowed us to study heterochromatin proteins in mammalian cell lines and in transgenic fruit flies. The results presented in thesis expand our knowledge of heterochromatin function and give new insights into the underlying mechanisms.