Investigating epigenome dynamics the RITE way
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SUMMARY

The DNA in the eukaryotic cell needs to be organized and packaged to fit in the limited dimensions of the nucleus and yet enable access to the genetic code. Packaging is done by wrapping the DNA around octamers of histone proteins, which are called nucleosomes. There are four canonical histone proteins: H2A, H2B, H3, and H4. A strand of nucleosomes is called chromatin. Over the last decade, it has become clear that chromatin is not a static structure that is only used by the cell as a packaging mechanism. In contrast, the structure of chromatin is very dynamic and has a profound influence on gene expression and other DNA transactions. Euchromatin, based on staining a chromatin state with a more open conformation, generally contains active genes and is more permissive for transcription. Closed chromatin, or heterochromatin, is usually silenced. Examples of heterochromatic regions in yeast are the silenced mating type loci and telomeres. The cell has several ways to alter chromatin structure. Histone proteins in the nucleosome can be modified by different post-translational modifications (PTMs) such as methylation, acetylation, or ubiquitylation. Some PTMs are connected with active chromatin, or gene activation. Others are associated with gene silencing and heterochromatin. Another way to alter chromatin structure is the incorporation of histone variants into the nucleosome. There are a number of different variants known; most variants make the nucleosome unstable, while one variant has been found that makes the nucleosome more stable. Chromatin structure can also be altered by remodeling the position and structure of nucleosomes across the genome, thereby modulating access of the underlying DNA to transcription regulators. A relatively unexplored layer of chromatin dynamics is histone exchange, that is, the replacement of a histone with the same type of histone without a prerequisite change in occupancy.

Chromatin is a very dynamic structure. In the Introduction and general discussion we discuss the findings described in this thesis on this topic. Histone exchange could have several critical functions in the cell. It can provide the cell with a means to modulate histone PTMs or to renew the chromatin and erase PTMs. Indeed, we established a link between one mark, H3K79me and histone stability. Histone exchange could also be a mechanism to expose or occlude transcription factor binding sites. Dynamic competition between nucleosomes and transcription factors for DNA binding suggests the possibility that histone exchange influences the transcriptional output of a transcription factor binding event. Histone exchange has been correlated to promoters and boundary regions, pointing towards histone exchange as a putative mechanism to prevent spreading of chromatin types. Recent studies have indicated that histone variant H3.3 is involved in key cellular processes such as epigenetic reprogramming and cancer development. Histone dynamics has also been linked to disease. Mutations in H3.3-deposition complexes have been found in human pancreatic neuroendocrine tumors that destabilize telomeres. In
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human glioblastomas, also mutations of H3.3 itself have been found. Interestingly, some of the mutations in H3.3 in these glioblastomas reduce global H3 methylation levels by inhibition of SET-domain enzymes, suggesting that the mutant H3.3 proteins act in a dominant manner to also influence canonical H3. These findings indicate that histone exchange plays a major role in gene expression and growth control and it will be important to elucidate the underlying mechanisms and to study the biological consequences when the dynamic behavior of histones is disturbed.

Whereas many of the previously mentioned dynamic changes in chromatin can be detected by conventional methods such as mapping histone PTMs or nucleosome occupancy across a genome under steady state conditions, to determine histone exchange, a distinction has to be made between the existing population of histones and the newly synthesized population. To this end, a number of different assays have been developed for use in yeast, Drosophila, slime mold, and human cells. In Chapter 1 an overview is given of the different methods to study histone exchange, as well as the results emerging from these studies. The pathways of the cell from the moment the histone proteins are produced to the assembly of nucleosomes are explained. Furthermore, the mechanisms of replication-coupled histone deposition and replication-independent histone exchange as they are known today are described. Also, an overview of histone variants and replacement of canonical histones by variants is delineated.

Chapter 2 highlights the dynamics of a particular histone PTM: methylation of lysine 79 on histone H3 (H3K79me). H3K79 can be mono-, di-, and tri-methylated and this epigenetic marking system has been implicated in mixed lineage leukemia and cell reprogramming. In this chapter, special attention is given to the association of H3K79me3 and ancestral histones, i.e. the inverse correlation between histone exchange and H3K79me3.

Histone exchange can only be determined when a distinction can be made between the old, existing population of histones and the newly synthesized population. We recently have developed a genetic pulse-chase assay called Recombination-Induced Tag Exchange (RITE). This assay employs an inducible switch from one epitope tag to another one to distinguish between old and new proteins. RITE has been successfully used in Saccharomyces cerevisiae to measure exchange and inheritance of histone proteins, to study changes in post-translational modifications on aging proteins, and to visualize the spatiotemporal inheritance of protein complexes and organelles in dividing cells. A series of successful RITE cassettes is described in Chapter 3. These cassettes have been designed for biochemical analyses, genomics studies, as well as single cell fluorescence applications. The use of these cassettes in immunoblotting, Chromatin Immunoprecipitation, and microscopy was shown.

Histone variants can replace canonical histones in the nucleosome, and have specific characteristics and functions. H2A.Z is a major variant of histone H2A, and has been implicated in various cell processes such as gene activation, chromosome
segregation, and cell cycle progression. In Chapter 4 the exchange of H2A.Z is described using the RITE assay. This variant is mostly located in promoter regions, where exchange of H3 is known to be high. Since H2A.Z destabilizes the nucleosome in vitro and is located in regions where histone H3 has a high level of exchange in vivo, we expected H2A.Z to be very dynamic. Surprisingly, our results showed that replication-independent exchange of H2A.Z is not higher than H3 exchange. Even overexpression of new H2A.Z did not completely remove all old H2A.Z from the chromatin. We hypothesized that this lack of detectable exchange is possibly caused by recycling of H2A.Z, and we are currently looking for the factors that might be involved in this process. Determining recycling is challenging as this goes undetected with most assays currently used to measure chromatin dynamics. To measure histone recycling, new methods will need to be developed that not only label old vs. new histones, but also evicted vs. not-incorporated histones.

In Chapter 5 we investigate the dynamics of H3K79 methylation by Dot1. Dot1 methylates H3K79 by a distributive manner, which leads to a changing pattern of mono-, di-, and trimethylation throughout the cell cycle. Using a mathematical model to describe the dynamic behavior of H3K79me, we predicted that the overall level of methylation of H3K79 increases over time. Indeed, when we arrested cells in G1, we saw an increase in H3K79me3. This led to the hypothesis that residence time of H3 in the chromatin is a determinant of the level of methylation. Using RITE to purify old H3 from dividing cells, we showed that H3K79 methylation indeed accumulates on old histones and that this accumulation can span multiple cell generations. This result is in contrast with the idea that cells re-establish the parental pattern of PTMs quickly after DNA replication, and makes it less likely that H3K79me has a role in epigenetic memory. Instead, this ongoing methylation could provide the cell with a timer mechanism to directly couple cell-cycle length to changes in chromatin modification on the nucleosome core.