Chromatin dynamics in yeast: The RITE assay for histone turnover and inheritance

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English Summary

Eukaryotic cells contain long strands of DNA that need to be packaged into small structures that fit in the limited space available in the nucleus of the cell. Packaging is done by wrapping the DNA around histone proteins, together called nucleosomes. The nucleosomes form a beads-on-a-string structure, known as chromatin, which can be further organized into higher order structures. Histones can be altered by proteins that add or remove chemical modifications to specific residues at specific positions. These histone modifications, which can occur in different combinations, can directly or indirectly affect the binding of regulatory proteins to chromatin. Thereby, they play an important role in gene regulation, DNA repair, and DNA replication. Histone modifications have also been suggested to act as epigenetic signals to facilitate the propagation of gene expression states. To maintain cell identity by chromatin-based mechanisms, daughter cells need to rapidly re-establish the parental epigenetic patterns following deposition of new unmodified histones on the duplicated DNA. Indeed, some modifications, such as histone methylation, are relatively stable and several copy mechanisms seem to be available. However, histones are not necessarily permanent residents in the genome once deposited during DNA replication. The first goal of the studies described in this thesis was to better understand the mechanisms by which histone modifiers that are associated with active regions of the genome affect the formation of silent chromatin domains. The second goal was to unravel the patterns and mechanisms of histone turnover and inheritance to study the role of histone turnover in epigenetic regulation.

Methylation of lysine 79 of histone H3 by Dot1 occurs in euchromatin (active regions) and promotes the formation of heterochromatin (inactive regions). The presence of this mark in euchromatin helps to prevent the non-specific binding of silencing proteins, which enhances binding at heterochromatin. By inactivating multiple other modifiers of euchromatin we found that they also affected heterochromatin and did so by mechanisms independent of Dot1 (chapter 2). Therefore, multiple histone modifications do not just act as local recruitment modules but can also act by more global and indirect means.

To start to investigate the role of histone turnover in epigenetic control, we developed a novel assay called recombination-induced tag exchange (RITE; chapter 3). This is a genetic pulse-chase tool that enables the discrimination between old and new proteins. RITE uses endogenous gene promoters and has the unique ability to switch epitope tags on a protein permanently, enabling the measurement of both loss of old as well as gain of new proteins. To measure histone turnover we applied RITE to histone H3. By comparing replication-independent to replication-dependent deposition we found that arrested cells can rapidly replace a large fraction of the genome-bound histones suggesting
that chromatin is a highly dynamic structure. Moreover, replication-independent turnover took place in all cell-cycle phases. By comparing the turnover at different loci in the genome we found that transcriptional activity of a gene is positively correlated with turnover and that induction of transcription causes histone turnover. We were also able to apply the RITE assay to the proteasome to study its stability, since it is known to be highly stable but unknown how it is degraded. We successfully added fluorescent proteins both before and after the switch to enable the use of microscopy.

To fully understand the relevance of a dynamic epigenome in which histone modifications can be constantly reset by turnover, players involved in histone turnover need to be manipulated. For that purpose we performed a screen to search for turnover factors (chapter 4). The screening tool we developed enabled the simultaneous measurement of histone turnover in many gene deletion mutants. We found several mutants that either positively or negatively affected histone turnover. The evolutionarily conserved histone acetyltransferase HAT-B complex was found to stimulate histone turnover, both through its acetyltransferase function as well as through its interacting factor Hif1. In addition, we found that Hat1 binds to a different subset of the pool of soluble histones than Asf1, indicating that Hat1 is mainly involved in assembly, whereas Asf1 mediates both assembly and disassembly of nucleosomes.

The dynamic behavior of histones challenges the idea that they may transmit cellular memory signals. To investigate the putative role of histones in memory, we used RITE to examine the inheritance of histones in replicating cells (chapter 5). To determine what happens to ancestral histones throughout cell division, the relative enrichment of old histones was determined genome wide after one, three and six cell divisions. We found that there are no large domains in the genome that retain old histones. Rather, they were predominantly retained at the beginning (5’ end) of most gene coding sequences (ORFs), with long genes and lowly transcribed genes accumulating more old histones than short genes or highly transcribed genes. Using a mathematical model that describes the observed data, several observations were made. 1) The relative enrichment of ancestral histone retention in cycling cells is not merely a result of replication-independent histone turnover. 2) During replication, the majority of histones spread around 400 bp from their original location. This, to our knowledge, is a first estimate for the distance of replication-coupled spreading in vivo. Our findings suggest that nucleosomes do stay close to their original location but are not copied to the same location with high precision. Therefore, chromatin seems to be a sloppy carrier of information if the information is carried on a single nucleosome. However, if information is present on multiple nucleosomes, i.e. a chromatin domain, histones may be well suited to pass on the histone signals to the daughter cells. 3) RNA polymerase II passage during transcription leads to a 100 bp lateral 5’ movement of nucleosomes. We expect
that by this retrograde movement of histones, histone modifications may also be transferred from 3’ to the 5’ end of a gene.

Histones are very dynamic and are inherited in specific patterns. To understand the biological significance of these processes it will be important to unravel the underlying mechanisms and study the consequences of disrupted histone dynamics. The screen that we developed and mutants that we already identified will be good starting points to study the significance of having balanced dynamic turnover and retention.