

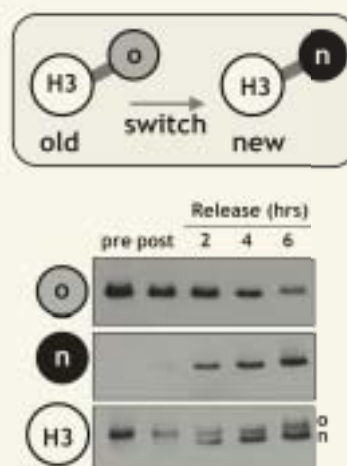
EPIGENETIC REGULATION OF GENE EXPRESSION

In eukaryotic cells the DNA is packaged into chromatin by histone proteins. Post-translational modifications of the histone proteins can affect chromatin structure and function and are involved in regulation of gene expression and DNA damage response. Changes in chromatin modification can also result in heritable changes in gene expression without changes in the actual genetic code and these epigenetic changes can lead to cancer. The mechanisms by which epigenetic imprints are established or prevented are still poorly understood. Many chromatin modifiers are conserved from yeast to humans. Our group uses the budding yeast *Saccharomyces cerevisiae* as a powerful model system to identify new epigenetic regulators and to unravel the molecular mechanisms by which chromatin-modifying enzymes affect chromatin structure, gene expression, and DNA damage response.

Function and regulation of histone H3 lysine 79 (H3K79) methylation by Dot1

We previously discovered a novel histone methyltransferase Dot1, which can add one, two, or three methyl groups to lysine 79 of histone H3 on the surface of the nucleosome core. Dot1, which is conserved from yeast to humans, influences heterochromatin structure and the DNA damage response, and has been implicated in oncogenic transformation in mammals. A major goal of our research is to understand how H3K79 methylation affects chromatin structure and function and how Dot1 is regulated. In contrast to other known protein methyltransferases, Dot1 acts by a non-processive mechanism. This unusual mechanism of synthesis affects the function of the methylated lysine and determines how it can be regulated. In general, different forms of lysine methylation have specific functions in chromatin. In contrast, the mono-, di-, and trimethylated forms of histone H3K79 showed functional overlap in promoting silencing, suggesting that the complex methylation pattern of H3K79 is read as a binary code of methylation or no methylation. In addition, whereas histone methylation typically acts as a binding signal, H3K79 methylation seems to act as an anti-binding signal. Since human and yeast Dot1 are structurally very similar, we expect that similar rules apply to human cells. Cell-cycle changes in H3K79 methylation in African trypanosomes are controlled by two Dot1 proteins that have distinct enzymatic activities.

Chromatin dynamics One of the main goals of our group is to understand how chromatin modifications can have long-term effects on gene expression. Post-translational modifications of histones have been proposed to be involved in epigenetic memory. When a cell divides, parental histones (containing the epigenetic marks) and newly synthesized histones (unmodified or in a ground state) are somehow assembled onto the daughter DNA strands in a manner that faithfully reproduces the transcriptional states of chromatin that existed prior to chromosome duplication. The exact mode of histone inheritance is still unclear and recent studies have shown that chromatin can be dynamic. We developed a novel assay in yeast to determine protein turnover *in vivo*. This universally applicable assay, called Recombination-Induced Tag Exchange (RITE), involves differential labeling of existing and newly synthesized proteins by a genetic epitope-tag switch system (see figure 5). Using RITE we found that histones throughout the genome are subject to extensive replication-independent exchange, suggesting that histones and their post-translational marks are not permanent residents in chromatin. We have recently developed high-throughput methods to identify proteins responsible for this mode of histone deposition and eviction. We expect that our studies will provide novel insights into the role of histones in maintaining and erasing established epigenetic patterns and gene expression programs.



Group leader Fred van Leeuwen

Fred van Leeuwen PhD Group leader
Iris Stulemeijer PhD Post-doc
Kitty Verzijlbergen MSc PhD student
Marit Terweij MSc PhD student
Hanneke Vlaming MSc PhD student
Tibor van Welsem Technical staff

Publications

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Figure 5: A genetic pulse-chase assay to determine protein turnover in vivo. The epitope tag on histone H3 can be switched at the genomic level from an old to a new tag in arrested yeast cells. Upon re-entry into the cell cycle old histone proteins disappear and new histone proteins accumulate.