Histone Modifications: Insights into Their Influence on Gene Expression

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This year's Albert Lasker Basic Medical Research Award honors David Allis and Michael Grunstein for their pioneering research that highlighted the importance of histones and their post-translational modifications in the direct control of gene expression.

Chromatin and Gene Expression

For over a century, it has been recognized that chromosomes in eukaryotes contain both DNA and protein, including histones. For some time, many believed that the protein components carried the inherited genetic information, but that all changed when the structure of the double helix of DNA was determined, revealing how information could be both stored and mutated as well as a general scheme for inheritance of genetic information.

Four and a half decades ago, Roger Kornberg proposed that chromatin consisted of a repeating unit of nucleosomes, which contained two copies of each of the four different histones with about 200 base pairs of DNA (Kornberg, 1974). High-resolution structures of a number of nucleosome particles have revealed the intimate details of the core particle, with the slightly twisted double helix making two turns around the core histone octamer particle $(H3{\cdot}H4)_2(H2A{\cdot}H2B)_2$. Histones with slightly different amino acid sequences can contribute to the diversity of nucleosome types and function within a single chromosome (Luger et al., 2012). For example, histone H3 can exist as H3.1 or H3.2 in most nucleosomes in a cell, but H3.3 is generally associated with transcribed DNA, and a related variant, CenH3, is found at centromeres, but these differences do not provide an explanation for how histones have a profound impact on nuclear function. Three years after the structure of the nucleosome was proposed, much discussion at a celebrated symposium on chromatin at Cold Spring Harbor Laboratory (during which RNA splicing was announced) focused on how histones and chromatin might affect gene expression. Ideas about the functions of histones ranged from passive structural participation in chromatin to an active role in repressing gene expression, but much of the discussion was speculative and inconclusive.

In pioneering research beginning in the early 1960s, Vincent Allfrey found that histones were modified by post-translational acetylation and methylation, and he correlated these modifications with control of gene expression (Allfrey et al., 1964). However, despite his extensive publications, these studies alone did not establish that these histone modifications were critical for the control of transcription. Allfrey died in 2002 and thus could not be considered for the Lasker Award. Building on these earlier correlative findings, research by David Allis and Michael Grunstein changed how we perceive the role of histones in control of gene expression and widened the appreciation of the intricacies and beauty of chromatin.

Gene Activation and Repression in Yeast

Grunstein demonstrated that histones played a major role in the activation of gene expression in the budding yeast *S. cerevisiae*. Initially studying the multiple histone genes themselves, Grunstein designed a yeast strain in which he could control the level of histone H4 in cells and showed that when histone H4 levels were reduced, some genes such as the highly regulated *PHO5* gene that is normally activated in the presence of low concentrations of inorganic phosphate had increased gene expression at normal phosphate levels (Han and Grunstein, 1988). Interestingly, not all genes responded the same way as *PHO5*, suggesting that nucleosomes at the promoter of *PHO5* repressed gene transcription. Consistent with this observation, Grunstein showed that the promoter was more accessible to nuclease digestion when histone H4 was lowered. This early study appeared to confirm what was already suspected, namely that nucleosome positioning over certain promoters could control the expression of a gene, consistent with the long-held view that histones in nucleosomes just got in the way of site-specific transcription factors and prevented access in a passive manner. But this view soon began to change.

Having mastered control of expression of the gene-encoding histone H4, Grunstein was able to make specific mutations that either eliminated or changed specific amino acids in the amino terminus of the histone H4 tail. He demonstrated that deletion of the histone H4 amino terminus, or more importantly mutation of the conserved lysine (K) residues that were known to be reversibly acetylated, affected induction of gene expression for highly regulated genes. In yeast with this mutated histone H4, Grunstein showed that activation of *GAL1* gene transcription in response to galactose and activation of the *PHO5* gene in low phosphate were significantly compromised (Durrin et al., 1991). Interestingly, not all genes were affected, since constitutively active genes were not altered in their expression. These results suggested that the lysine residues in histone H4 and possibly their acetylation were required for induction of gene expression.

Another well-studied and interesting gene regulatory system in budding yeast was the heritable repression of the silent

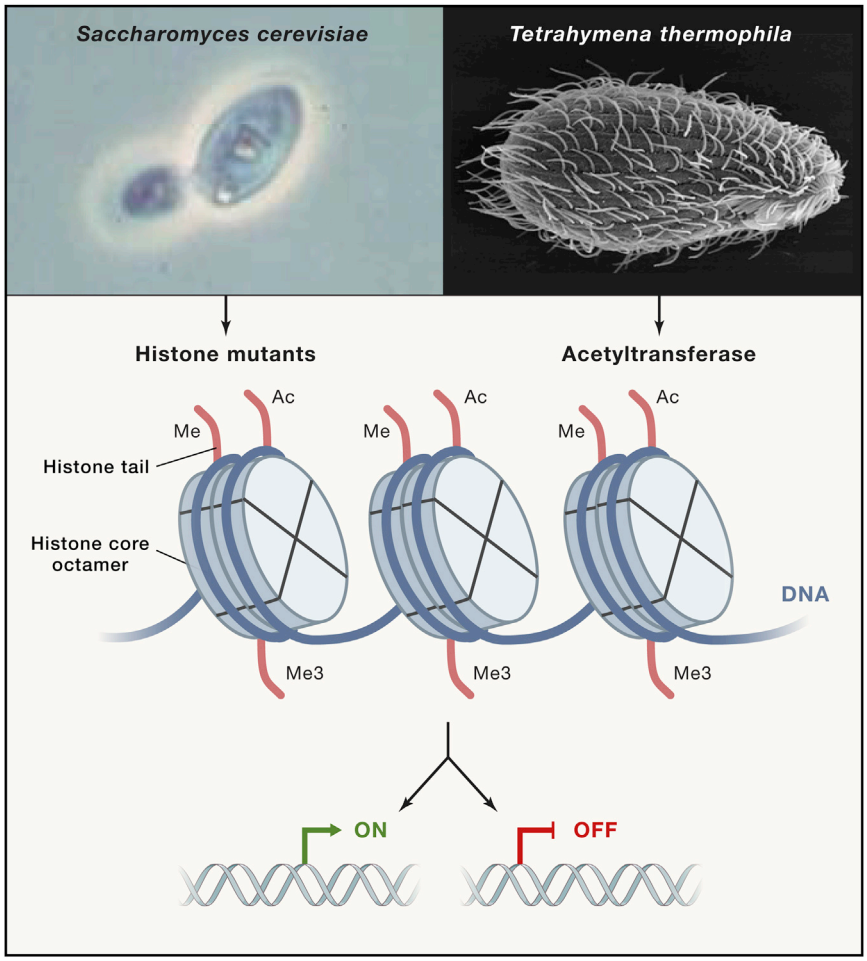

Figure 1. Histone Modifications and Gene Expression

Top: Studies in two model organisms, *S. cerevisiae* and *Tetrahymena*, resulted in the discovery of the importance of histones in nucleosomes for both activation or repression of gene expression via the discovery of enzymes that can post-translationally modify histones at specific, non-random sites (pictures not to scale). Middle: The histone $(H3 \cdot H4)_2(H2A \cdot H2B)_2$ core octamer and the histone tails in nucleosomes can be modified at specific sites, and the modification writers can spread these marks to adjacent chromatin. Bottom: Histone modifications can either activate (green) or repress (red) gene transcription.

mating type loci *HMLα* and *HMRa*. These genes are stably repressed by the action of silent information regulator proteins SIR1, SIR2 (now known to be an NAD-dependent histone deacetylase), SIR3, and SIR4. Grunstein demonstrated that the histone H4 amino-terminal lysine residues, particularly K16, are required for silencing of *HMLα* and *HMRa* gene transcription (Johnson et al., 1990). Extragenic suppressors of the histone H4K16G (lysine to glycine) mutation mapped to the *SIR3* gene, but these *sir3* mutations could not suppress a deletion of the histone H4 tail. He later showed that histones H3 and H4 interacted with SIR3 and SIR4. The SIR3 and SIR4 proteins are also involved in repression of transcription at telomeres, and at the highly repeated ribosomal genes, and spread along chromatin by interactions with sequence-specific DNA-binding proteins and with histones (Hecht et al., 1996). Thus, Grunstein's research opened the door for an acceptance of a critical role for histones, and particularly the histone amino-terminal tails, in control of gene transcription: both regulated activation and inherited gene silencing. Importantly, the focus on the lysine residues in the amino-terminal tail of histone H4 provided a possible link to histone acetylation in the regulation of gene transcription (Figure 1).

The Macronucleus of *Tetrahymena* Yields Insights

In parallel to these studies in yeast, studies of another model organism, *Tetrahymena*, provided great insight into the role of histones and their post-translational modifications. David Allis investigated the site-specific acetylation of histones in both the *Tetrahymena* macronucleus, where genes are expressed, and in the micronucleus, which is equivalent to the germline of *Tetrahymena.* The histone H3 and H4 amino-terminal tails harbored non-random acetylation of lysine residues that were specific for the macronucleus (Chicoine et al., 1986).

A major surprise and key turning point in the field of chromatin and gene regulation came in 1996, when David Allis reported the purification of the *Tetrahymena* type A histone acetyltransferase and showed that it was the homolog of the yeast transcriptional co-activator protein GCN5 (Brownell et al., 1996) (Figure 1). He also demonstrated that yeast GCN5 harbored histone acetyltransferase activity. Allis soon showed that GCN5 acetylated specific lysine residues in the tails of histones H3 and H4, which were distinct from the lysines acetylated by the type B histone acetyltransferase and associated with the deposition of newly synthesized histones during DNA replication (Kuo et al., 1996). Allis also recognized that the enzymes acetylated lysines within a specific sequence of amino acids in the histone tails, analogous to the substrate specificity of protein kinases for particular amino acid sequence motifs. The observations by Allis and colleagues galvanized the field of transcription and focused considerable attention on the role of histone modifications in the control of gene transcription. The spigot was now turned on full—other histone acetyltransferases related to transcription factors quickly emerged from the Allis lab and others.

Just as phosphatases can reverse the phosphorylation of proteins, histone deacetylases were discovered that could reverse histone acetylation. Teruhiko Beppu and colleagues discovered (R)-trichostatin A (TSA), a natural product from *Streptomyces* that inhibits cell proliferation and differentiation in mammalian cells and increases histone acetylation *in vivo* (Yoshida et al., 1990). Cells that were resistant to TSA did not accumulate

increased levels of acetylated histones and harbored a histone deacetylase that was 10-fold more resistant to the drug than the histone deacetylase from TSA-sensitive cells. Furthermore, Beppu identified trapoxin, a fungal-derived cyclo-tetrapeptide that could also increase histone acetylation *in vivo* and inhibit a histone acetyltransferase (Kijima et al., 1993). Later, Stuart Schreiber identified this enzyme from human Jurkat T cells and revealed it to be very similar to the yeast transcriptional regulator RPD3 (Taunton et al., 1996). These studies contributed links between reversible histone acetylation and transcriptional gene regulation.

An Explosion of Modifications

With Thomas Jenuwein, Allis identified the first histone methyltransferase that could methylate specific residues in the histone tails, setting off a cascade of discoveries of many such enzymes and eventually the discovery of interesting histone demethylases (Shilatifard, 2006). The flood gates had opened, and soon Allis and others were discussing a "histone code," read by proteins that bound to specific modifications of histones (Strahl and Allis, 2000). It is now known that a plethora of post-translational modifications are present on histones, including acetylation, methylation (mono-, di-, and tri-methylation on single residues, each playing different roles), phosphorylation, ADP-ribosylation, and ubiquitination, as well as lesser-studied modifications such as succinylation, butyrylation, and many more (see a remarkable chart of these modifications in the SnapShot [Huang et al., 2014]). In the strict sense, a code that can be deciphered does not appear to exist, but certain histone modifications are enriched in either actively transcribed or repressed regions of chromatin.

Allis developed many of the antibody reagents that recognize specific modifications at particular sites in histone proteins and used these to examine their presence, association with other proteins, and rates of turnover. Grunstein employed such antibodies in a technology called chromatin immunoprecipitation (ChIP) coupled with polymerase chain reaction detection of the associated DNA to map acetyl-lysine modifications to specific sites within the yeast genome. Now, ChIP coupled with DNA sequencing (ChIP-seq) is widely employed to study the biology of many types of histone modifications.

Since the pioneering work of Allis and Grunstein, there has been a flood of research on chromatin modifications and their influence on fundamental cellular processes such as nuclear chromosome structure and organization, gene transcription, DNA replication, and DNA repair. Moreover, the very abundant histones with the numerous nucleosomes in a cell's nucleus are substrates for highly dynamic acetylation and de-acetylation reactions. The principal donor for all of these acetyl marks, acetyl-coA, is also required for cellular metabolism, including the synthesis of carbohydrates and lipids, and for the cell's energy output. Thus, cell metabolism and histone modifications are ineluctably linked.

There are now known to be over 100 "reader" proteins that recognize histone marks. Potentially there are about 50 "writers" that create the modifications and about a dozen enzymes that can remove the histone modification marks, often called "erasers." This diverse set of proteins has been implicated in many diseases, and mutations in the set are often found in cancers, a focus of more recent research from the Allis laboratory and many others. Many of the writer enzymes are now drug targets for a variety of diseases. Understanding this group of readers, writers, and erasers has become important for all of biology, particularly for development.

Lessons

There are many important lessons from the research conducted by Grunstein and Allis, but perhaps the most important is that studies of model organisms such as the budding yeast *S. cerevisiae* and *Tetrahymena* yield insights into biology that can have a very broad impact, in this instance creating an entire field of investigation that touches on nearly all aspects of biology and medicine. Allis's studies on *Tetrahymena* also teach another lesson: that certain organisms have unique biological processes—such as creation of a macronucleus—that facilitate discoveries fundamental to an understanding of how gene expression is regulated in all organisms. Indeed, the same biological system was used to discover telomerase. Investigating biological diversity just because it exists is well worthwhile, for such studies may open the door to make groundbreaking discoveries. A final point is that both Allis and Grunstein have been supported by public, taxpayer funds from the National Institutes of Health, strengthening the argument that these funds should continue to promote investigator-initiated and curiosity-driven basic research on model organisms, leading—via paths unpredictable—to major discoveries.

David Allis and Michael Grunstein have made key contributions to many areas of biology, and their discoveries on chromatin and histone modifications have resulted in an explosion of research that has transformed understanding of the control of gene expression across eukaryotes. While many others have made major contributions to our current knowledge, the early insights of these two outstanding scientists make them deserving recipients of the 2018 Albert Lasker Basic Medical Research Award.

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