Meeting report
The nucleosome: from wallflower to Queen of the Ball
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A report on the FASEB meeting on Chromatin and Transcription, Snowmass, USA, 7-12 July 2001.

Ten or twelve years ago, when nearly every issue of *Science* and *Cell* featured a new insight into the molecular mechanisms of the eukaryotic cell cycle, nobody cared much about that lowly repeat unit of chromatin structure, the nucleosome. She was drab, embarrassingly nondescript, like dozens - no thousands - of others, and certainly unlikely to be light on her feet. The nucleosome was the proverbial 'wallflower', not one to attract attention. But she had her believers: a few chromatin-zealots realized that those beautiful dancing chromosomes - and much of gene transcription - might reduce to a thorough understanding of the basic structural unit that organizes DNA. Well, at the FASEB Meeting on Chromatin and Transcription, which took place on 7-12 July this year, with record participation, the one-time wallflower became Queen of the Ball. There was not a session in which at least one of the more brilliant speakers did not take her for a spin. Revealing hidden treasures of modifications and add-ons, the nucleosome definitely blossomed.

As with most beautiful objects, it is not the generality of the nucleosome but the tiny little details that count. Nearly 30% of the protein mass of the nucleosome does not participate in its hydrophobic core, which forms the stable bead around which DNA wraps twice. These 30% represent the amino- and carboxy-terminal tail domains, differing in length and composition for each of the four histones, yet rich with charged amino acids and sites for post-translational modification (Figure 1). For the most part, the histone tails are too mobile to figure in the crystal structures of the nucleosome, whether it is the original published by the Richmond laboratory in *Nature* in 1997 or the variant structures, as presented at the meeting by Karolin Luger (Colorado State University, Fort

Collins, USA). This reinforces our image of histone tails as roughly equivalent to the arms and legs of a human body: long and supple, just perfect for making contact with other bodies.

Despite many other exciting talks at the FASEB meeting, the more memorable ones concerned the functional implications of histone-tail modification and these are described below. The field has progressed significantly beyond the stage of compiling inventories. Direct correlations between particular post-translational modifications, such as acetylation or methylation, and the activation or repression of promoters were presented. Moreover, new data define a hierarchy of steps, often involving sequential modifications of the same lysine residue, that shift a promoter from an open to a repressed chromatin state. In many cases these changes are interdependent, progressive and conserved. Such observations suggest a mechanistic view of promoter activation as the 'mobilization' or 'exposing' of a particular nucleosome or nucleosomal parts, with repression being just the opposite. To add interest, the field has started to identify the proteins that recognize uniquely modified histone tails and the enzymes responsible for these modifications. These are exciting times. A few of the more significant talks are summarized below.

Shelley Berger (Wistar Institute, Philadelphia, USA) presented work on the regulation of the yeast *INO1* promoter, which in its active state contains several nucleosomes in which histone H3 is both phosphorylated on serine 10 (S10) and acetylated on lysine 14 (K14). This was demonstrated using chromatin immunoprecipitation (ChIP) with an antibody that specifically recognizes only the tails carrying both modifications at once. The complexes responsible for these modifications were then purified, identifying the kinase that targets S10 as the product of the *SNF1* gene. Previous work had shown that the histone acetyl transferase responsible for K14 modification of H3 is encoded by *GCN5*. By using
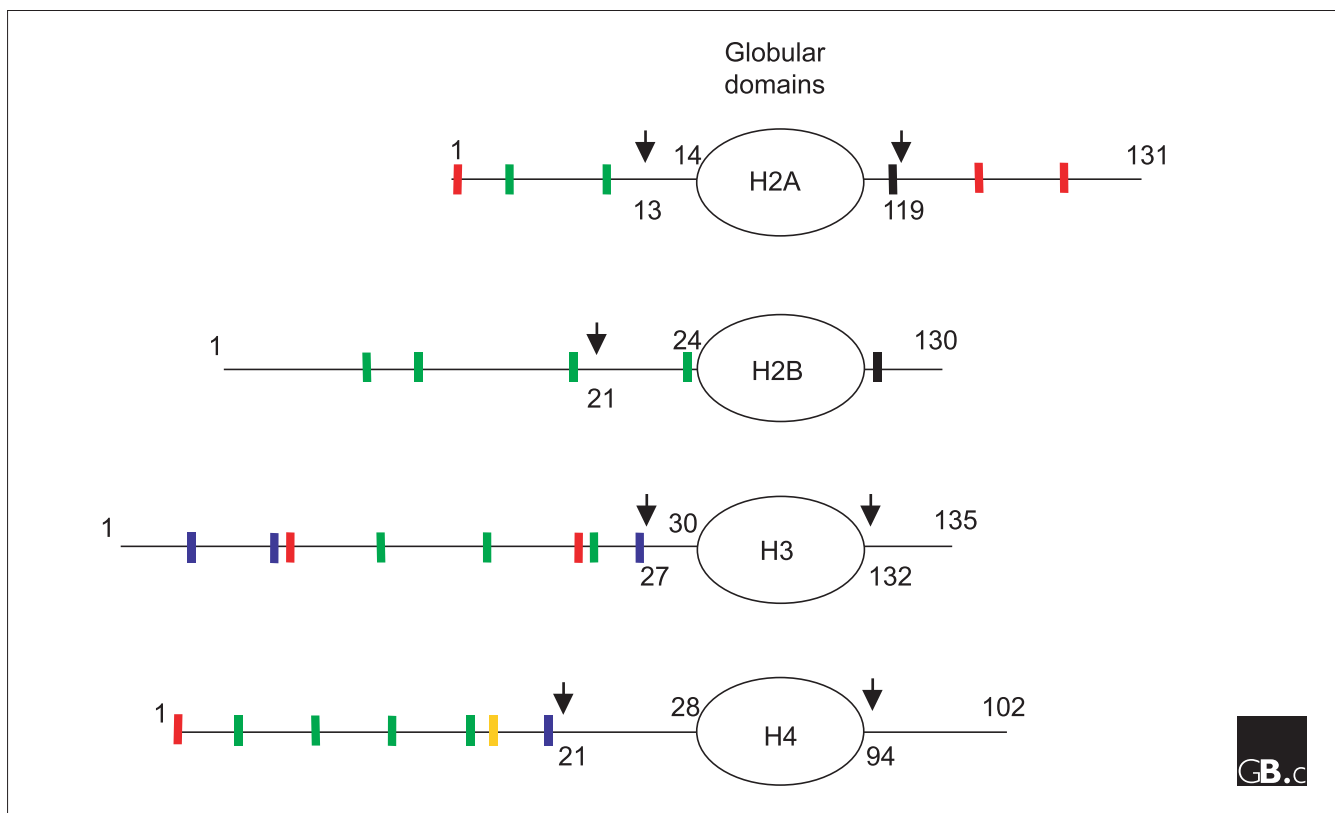
Figure 1
Histone cores and tails. Here, we indicate the inner ends of the histones, amino- and carboxy-terminal tails, which lie a few amino acids towards the core domain from the indicated trypsin cleavage sites (black arrows), using the amino-acid numbering from yeast. Note that this criterion minimizes tail size slightly, as some modifications and some partially extended or flexible structure is found internal to these sites (within the 'globular' domains). Potentially charged residues and target sites of modification are indicated with bars (green for a lysine that is only known as an acetylation target; blue for a lysine that can be either acetylated or methylated; black for a lysine that can be ubiquinated; red for a phosphorylatable serine or threonine; yellow, a histidine that can be phosphorylated). Modified arginines and potential acceptors of ADP-ribose, Sumo and/or ubiquitin are not indicated.

mutants lacking these enzymes, Berger's laboratory has found that phosphorylation on S10 is a prerequisite for acetylation on K14. Indeed, when serine 10 is mutagenized to alanine or the Snf1 kinase is deleted, acetylation of H3 K14 no longer occurs. On the other hand, loss of the Gcn5p acetyl transferase does not affect phosphorylation of S10. These data reveal a step-by-step activation mechanism in which the phosphorylation on S10 occurs first, promoting acetylation of K14 in turn. Intriguingly, Snf1 kinase activity is specific for a certain number of promoters but does not affect all those sensitive to the Gcn5p histone acetylase, implying the existence of other S10 kinases. This allows for differential activation of genes through the same set of modifications.

A further breakthrough has been in the study of lysine methylation. Unlike acetylation, which alters the charge of the histone tail, methylation leaves the ε amino group of lysine positively charged. This modification affects both lysines 4 and 9 of the histone H3 tail, but, remarkably, these two methylation events have opposite effects on the activity of the promoters and chromosome domains tested. Using antibodies specific for the methylated form of K4 or K14 of H3, the collaborators of David Allis (University of Virginia, Charlottesville, USA), Geneviève Almouzni (Institut Curie, Paris, France) and Thomas Jenuwein (Institute for Molecular Pathology, Vienna, Austria) have jointly established two strong correlations: one between the active chromatin state and methylation of H3 on K4, the second between a repressed chromatin state and methylated K9 of H3. Moreover, these correlations have been demonstrated both in organisms as diverse as fission yeast (Shiv Grewel, Cold Spring Harbor Laboratory, USA; reported by David Allis) and man. The antibody recognizing the methylated H3 K4[me] stains euchromatin, whereas that specific for H3 K9[me] stains the inactive X chromosome (the Barr body) in interphase. The Jenuwein lab has raised an alternative antibody against a branched form of H3 K9[me] (which has multiple linked lysine residues), and this antibody additionally stains the

nucleolus in interphase and centromeres in mitosis. The difference between the two H3 K9^{me} antibodies is proposed to reflect differences resulting from a higher-order folding of the chromatin fiber in heterochromatic regions. All groups agree that the H3 K9^{me} tag correlates strongly with a transcriptionally inactive state. ChIP analysis, for example, shows that H3 K9^{me} and H3 K4^{me} are associated, respectively, with the promoters of inactive and active genes (*PGK1* and *Xist*). A sharp transition between two regions containing one or other modification was also evident in the ChIP data presented by Gary Felsenfeld (National Institute of Health, Bethesda, USA): the H3 K9 methylation that is found in flanking regions stops precisely at the boundary element of the active chicken β-globin locus, being replaced by H3 K4^{me}, which increases sharply across the active promoter.

In human cells, one enzyme responsible for methylation of K9 is Suv3-9H1, which contains a SET domain, a feature that characterizes a subset of chromatin modulators. There are homologs in *Drosophila* and the fission yeast, named Su(VAR)3-9 and Clr4p, respectively, and mutation of either gene abolishes the staining observed with the antibody to H3 K9^{me} and reduces heterochromatin-mediated gene repression. Indeed, the methylation of H3 K9 by Su(VAR)3-9 creates a binding site for the heterochromatin protein HP1 (called Swi6p in *Schizosaccharomyces pombe*), which can

promote a stably repressed state of chromatin. This pathway is not the unique means through which the modification acts, however, for repression through H3 K9^{me} on the inactive X chromosome is independent of HP1 binding.

The laboratory of Tony Kouzarides (Wellcome Trust/CRC Institute, Cambridge, UK) has shown by ChIP that in mammalian cells the recruitment of HP1 to H3 K9^{me} depends on the retinoblastoma (Rb) protein, which is itself bound to promoters through the transcription factor E2F. Rb protein in turn recruits first the NuRD complex of proteins, which is able to deacetylate histone H3 K9 through its subcomponent histone deacetylase 1, a step that is likely to precede methylation. Then Rb recruits Suv3-9H1, which methylates the same lysine, thereby creating a binding site for the heterochromatin-associated protein HP1. In these promoter-specific repression events, it is not clear whether HP1 spreads along nucleosomes or represses locally. In contrast, methylation of H3 K4 is due to the product of the *SET1* gene (reported by both Allis and Kouzarides), which is conserved from budding yeast to man. H3 K4 methylation may prevent the recruitment of the NuRD complex, ensuring that H3 K9 stays acetylated, and the combined acetyl K4/K9 apparently activates transcription. This series of events provides a means through which histone H3 K4 and K9 methylation remain mutually exclusive, rendering chromatin stably
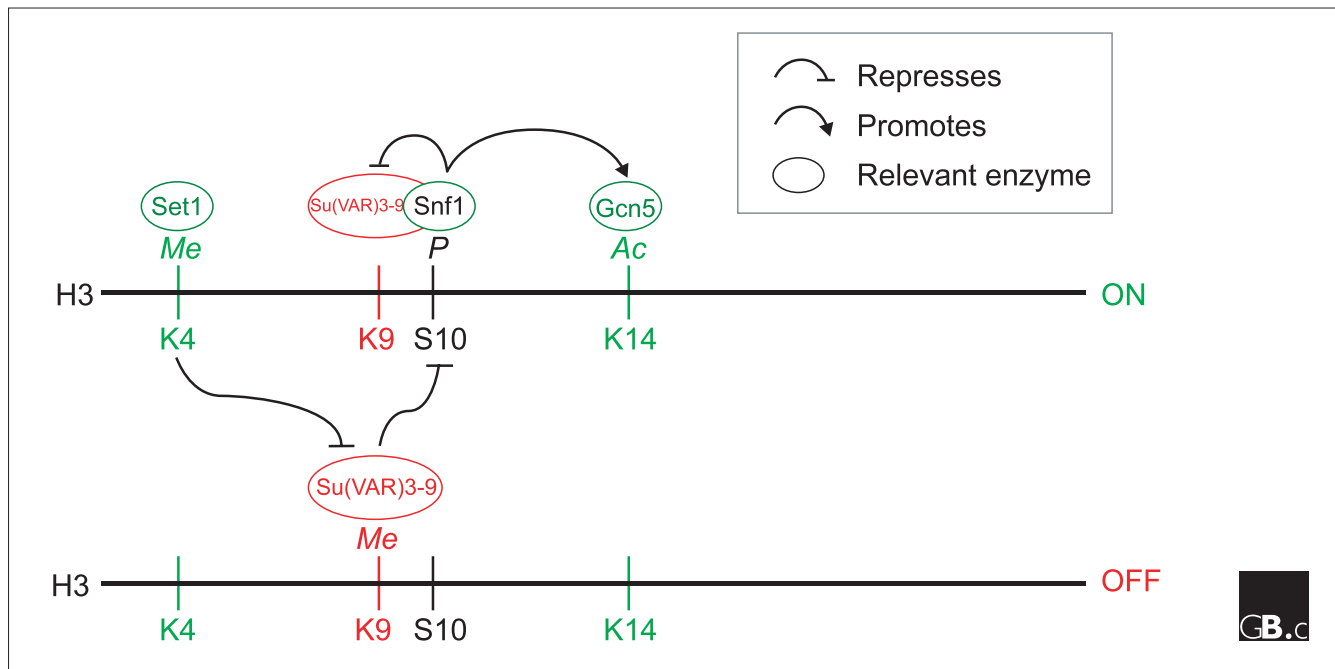
Figure 2
Modifications of the histone H3 amino-terminal tail. Modifications that correlate with transcriptional states are indicated here as either 'on' or 'off'. The enzymes (either in yeast, *Drosophila* or human) known to modify the given sites are indicated in ovals. Green indicates activating enzymes or modifications and red repressive modifications or enzymes. S10 phosphorylation can be present in both repressed and active domains. See text for further details. Ac, acetylation; Me, methylation; P, phosphorylation.

'open' or 'closed'. The various modifications of histone H3 amino-terminal tails are illustrated in Figure 2.

The nucleosome's most brilliant jewel this year was the amino-terminal tail of histone H3, yet new data on the ubiquitination of the histone H2B carboxy-terminal tail, and phosphorylation of the H2A tail, were presented by Mary-Ann Osley (Sloan-Kettering Cancer Center, New York, USA) and Art Lustig (Tulane University, New Orleans, USA), respectively. Mutation of the ubiquitinated lysine in H2B showed a synthetic effect with mutations in chromatin-remodeling enzymes, linking ubiquitinated H2B to transcriptional activation. Lustig found that H2A carboxy-terminal phosphorylation, on the other hand, influenced the efficiency of DNA repair. It is likely that most of the histone tails will participate in chromatin signalling, each modification maintaining a special relationship to other modifications within the same tail. As previously discussed, if one starts to compound the possible interdependencies, including *cis*- and *trans*-tail cross-regulation of the six histone modifications we know to date (acetylation, phosphorylation, methylation, ribosylation, sumolation and ubiquitination), the histone tail language becomes rich and entertaining. Like the cell cycle 10-12 years ago, the field is ripe for imaginative minds and further work.