Meeting report

Histones: should I stay or should I go?

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Published: 14 January 2005

Genome ***Biology*** 2005, **6:**306

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2005/6/2/306

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A report on the American Society for Biochemistry and Molecular Biology symposium 'Transcriptional Regulation by Chromatin and RNA polymerase II', Lake Tahoe, USA, 29 October-1 November 2004.

This year's American Society for Biochemistry and Molecular Biology symposium on transcription covered a wide variety of topics ranging from chromatin regulation, through the initiation of transcription and elongation process during transcription by RNA polymerase II (Pol II) to the roles of signaling in transcription and development. This report focuses on the sessions on chromatin, which led to many insightful discussions as a consequence of the rapid advances in this field over the past few years.

Direct and indirect functions of histone modifications

A central role for chromatin in regulating processes such as transcription and replication is now widely recognized. It is generally believed that the dynamic regulation of chromatin structure makes use of a diverse repertoire of post-translational histone modifications, ATP-dependent chromatin remodeling and histone-variant exchange. Prevailing views on histone modification and its consequences for the modulation of chromatin dynamics, as proposed in the 'histone code hypothesis', suggest that combinations of covalent modifications of specific histone residues comprise a structural and chemical 'code' that can be recognized by other protein modules which then regulate DNA accessibility and function.

Tony Kouzarides (University of Cambridge, UK) presented an interesting case that strongly supports this hypothesis. His group has found that methylation of lysine 20 of histone H4 in the fission yeast *Schizosaccharomyces pombe* is mediated by a novel protein, Set9, that contains a SET domain, a feature that characterizes a subset of chromatin modulators. Unlike other cases of histone lysine methylation, H4 Lys20 methylation appears not to be involved in regulating transcription or the formation of heterochromatin. Instead, it plays a pivotal role in the DNA-damage response pathway. Loss-of-function Set9 mutants and histone H4 K20R (lysine to arginine) mutants prematurely proceed to mitosis by skipping through the checkpoint that can cause arrest between G2 and M phases, even if they have unrepaired DNA. Kouzarides reported that Set9 is required for phosphorylation of the cell-cycle checkpoint protein Crb2, a homolog of the mammalian p53-binding protein 53BP1, and its recruitment to DNA double-strand breaks. He proposed that Crb2 might bind to methylated histone through its Tudor domain, which is present in many RNA-binding proteins. He made the interesting point that the H4 Lys20 methylation itself was not induced upon DNA damage and thus must be present already. Given that Crb2 binds to methylated H4 only at double-strand breaks, it was suggested that high-order structural change in chromatin elicited by DNA damage might be crucial for exposing the buried modified histone tails to their binding partners.

Recent systematic proteomic studies have identified the physical location of a number of novel histone modifications, and Michael Cosgrove (Johns Hopkins School of Medicine, Baltimore, USA) called our attention to certain scenarios in which dynamic chromatin regulation might not be explained by the histone code hypothesis alone. Cosgrove has mapped known histone modifications onto the crystal structure of the nucleosome core particle and he pointed out that a good proportion of them lie in the globular domains of the core histones. In fact, many modifications are positioned at the nucleosome lateral surface, where they would be likely to

affect histone-DNA interactions. From a structural perspective he suggested that these modifications might have direct roles in fine tuning histone-DNA contacts. Moreover, for these modifications to be made to pre-assembled nucleosomes, a dramatic conformational change would be required to make specific residues accessible to the modifying enzyme.

Cosgrove proposed a 'regulated nucleosome mobility' model, in which the mobility of nucleosomes is dictated by the affinity of histone octamers for DNA, which is in turn highly regulated by the concerted action of ATP-dependent remodeling factors and histone-modifying enzymes. In this model, remodeling and histone modification can mutually influence each other; this differs from the predictions of the histone-code hypothesis that histone modifications are established first and then direct later remodeling events. The eventual changes in nucleosome mobility can result from either remodeling or covalent modifications or combinations of both. Cosgrove cited genetic evidence to support this argument, noting that some *Sin* (Swi-independent) mutations in histones, which can bypass the requirements for Swi/Snf chromatin-remodeling enzymes or the histone acetyl transferase Gcn5 for transcription, coincide with several modified residues located at histone-DNA contact regions, suggesting that it is nucleosome mobility that matters.

Nucleosome displacement in transcription

Several presentations at the meeting shed new light on the relationship between nucleosome occupancy and transcriptional activity. In a poster presentation, Cheol-Koo Lee of Jason Lieb's laboratory (University of North Carolina, Chapel Hill, USA) described their work on determining relative nucleosome occupancy throughout the *Saccharomyces cerevisiae* genome. Using chromatin immunoprecipitation (ChIP) assays in combination with DNA microarrays that cover the entire yeast genome at a 1 kilobase (kb) resolution, they found that immunoprecipitating Myc-tagged histone H4 and histone H3 preferentially pulls down more coding DNA than non-coding DNA. This suggests that nucleosomes are depleted from active regulatory elements. By comparing their data to transcription-profile datasets, Lee and colleagues found that the extent of nucleosome occupancy at gene promoters is inversely proportional to the rate of gene transcription. Moreover, when growth conditions are altered, nucleosome occupancy on promoters undergoes some dramatic changes as gene-expression patterns change, but the same inverse correlation still applies. Interestingly, this universal pattern of nucleosome distribution does not appear to rely on specific histone tails, as Lee and colleagues observed similar results in yeast strains bearing various tail-less histone mutations.

Kevin Struhl (Harvard Medical School, Boston, USA) reached a similar conclusion - that there is an inverse correlation between nucleosome occupancy and transcription - for the yeast *GAL1* promoter. He also reported that heat inactivation of the Pol II carboxy-terminal domain kinase Kin28 caused a reduction of Pol II occupancy at promoters and coding regions, and a corresponding increase in H3 occupancy of the same regions. This suggests a tight link between the presence of Pol II and histone loss. Using an artificial gene containing a *GAL1* promoter driving a long coding sequence, Struhl and colleagues were able to monitor the last wave of Pol II traveling along the gene after inactivation of the promoter. They discovered that as Pol II transcribed through the gene, histones were immediately deposited onto the DNA behind the polymerase, thus restoring the chromatin structure.

Struhl also presented biochemical data suggesting that promoter DNA may resist nucleosome formation. He and his colleagues reconstituted nucleosomes with yeast genomic DNA *in vitro*, isolated the free DNA from the assembled nucleosomes, and subsequently hybridized it to DNA microarrays of the whole yeast genome. They found that 75-80% of promoter regions were half as likely to reconstitute nucleosomes than were coding regions. This suggests that intrinsic properties of promoter DNA sequence may contribute to nucleosomal distribution in a manner independent of transcription.

David Gross (Louisiana State University Health Sciences Center, New Orleans, USA) has also observed apparent histone eviction in yet another model system. Heat-shock activation of the *S. cerevisiae HSP82* gene coincided with loss of nucleosomes from its promoter and coding regions. He claimed that dissociation of histones at *HSP82* was not caused by the global effects of heat shock, as two genes (*YAR1, CIN2*) adjacent to *HSP82* did not lose histones. Unlike the scenarios described by Lee, Gross observed a transient hyperacetylation of H2A, H3 and H4 tails at promoter regions prior to the loss of histones; the functional consequence of this is still unclear, however. Most importantly, in this case transcription *per se* does not seem to be the direct cause of these dramatic changes in the chromatin. Deletion of the TATA box reduced *HSP82* transcription to a much lower level than that observed with a promoter mutation that weakens binding of the transcriptional activator heat-shock factor (HSF). Nevertheless, the domain-wide histone eviction is almost unaffected in the TATA mutant but is abolished when HSF can no longer efficiently bind to the upstream sequence. This is in marked contrast to Struhl's results, where transcription seems to have an active role. What causes histone dissociation at *HSP82* is largely unknown; dissociation does, however, seem to be independent of some prominent chromatin remodelers such as Swi/Snf, Gcn5, the histone methylase Set1 and elongation factor Paf1.

From studies to determine which factors might be directly responsible for nucleosome loss, Melissa Adkins of Jessica

Tyler's lab (University of Colorado Health Sciences Center, Denver, USA) provided evidence that the histone H3/H4 chaperone Asf1 mediates the loss of nucleosomes at the *PHO5* and *PHO8* promoters upon activation and that histone loss was essential for transcriptional activation of these two yeast genes. This observation implies that in this case histone eviction might not be driven by the Pol II machinery traveling along the DNA; rather, it might be a prerequisite for Pol II transcription to occur after the activator (Pho4) has bound. Adkins also reported that nucleosomes were reassembled onto the *PHO5* promoter during gene repression and that the protein Spt6 might be involved in this reassembly. This is consistent with the known role of Spt6 in repressing faulty transcription from cryptic start sites in coding regions. Unsurprisingly, however, Asf1 cannot be the single answer for all genes because it seems to be unnecessary for the activation of *GAL1* and *HSP82*.

The apparent discrepancies among these reports may very well reflect the diversity and subtlety of chromatin regulation at different genes, or we may simply be missing some important links in our understanding. It is clear, however, that great strides have been made in establishing the paradigm that assembly and disassembly of nucleosomes occurs in a very dynamic manner even within a single cell cycle.