Meeting report

Chromatin meets RNA polymerase II

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A report on the Cold Spring Harbor Laboratory meeting 'Mechanisms of eukaryotic transcription', Cold Spring Harbor, USA, 2 August-2 September 2007.

It is becoming increasingly clear that the mechanisms governing eukaryotic transcription are as diverse and complex as the organisms in which they are studied. The recent Cold Spring Harbor Laboratory meeting on mechanisms of eukaryotic transcription covered various topics, including epigenetics, the architecture and regulation of the chromatin landscape through histone modifications, and the mechanisms of transcription initiation and elongation by RNA polymerase II (Pol II). Here we report on the latter two topics, attempting to integrate chromatin and Pol II regulatory mechanisms.

Chromatin architecture and histone crosstalk networks

How nucleosomes are organized throughout a genome sets the stage on which the transcription machinery engages each and every gene. Complete high-resolution maps of nucleosome locations and their modifications are now coming to light through high-resolution and data-intensive technologies such as chromatin immunoprecipitation followed by sequencing or microarray identification of the pulled-down DNA (ChIP-seq or ChIP-chip, respectively).

Keji Zhao (National Heart, Lung, and Blood Institute, NIH, Bethesda, USA) reported work on the genome-wide distributions of numerous histone modifications and the histone variant H2A.Z in human cells. Using ChIP-seq, he found that monomethylation of H3 lysine 9 (K9), H3K27, H3K79, H4K20, and H2BK5 is associated with gene activation, whereas trimethylation of H3K9, H3K27, and H3K79 is linked to repression. He also found that H2A.Z was preferentially found in promoter regions, consistent with previous reports in yeast. One of us (B.F.P.) reported genome-wide maps of nucleosome locations in *Saccharomyces cerevisiae* and *Drosophila melanogaster* obtained using ChIP-seq. Their nucleosome organization was found to be remarkably similar in many respects, including nucleosome-free regions at the beginning and end of genes. However, flies place their +1 nucleosome (the first nucleosome downstream of the transcription start site) a bit further downstream than in yeast, which leaves the transcription start site intrinsically accessible in flies.

The chromatin landscape is peppered with numerous posttranslational modifications to histone tails, which serve in part to regulate Pol II as it engages and traverses genes. Recent evidence has begun to delineate the regulatory pathways controlling several histone modifications. The Bur1-Bur2 kinase regulates Set2-catalyzed methylation of H3K36 in the coding regions of transcriptionally active genes, which can serve as a mark to recruit the Rpd3 histone deacetylase (HDAC). Consequently, this pathway establishes an inverse relationship between the levels of H3K36 methylation and histone acetylation at some genes. Karen Arndt (University of Pittsburgh, USA) reported that yeast Paf1 plays a role in regulating the trimethylation levels in this pathway. Using ChIP assays in *paf1* deletion and *bur1* deletion mutants, she found that trimethylated H3K36 is preferentially decreased at the 5' ends of genes in these mutants, whereas acetylation of H3 and H4 is increased. Arndt proposed that the Bur1-Bur2 kinase functions upstream of the Paf1 complex to regulate H3K36 trimethylation and H3 and H4 acetylation at the 5' ends of genes.

To prevent spurious initiation by Pol II within the coding region, the Rpd3 complex (Rpd3S) maintains hypoacetylated coding regions by recognizing the H3K36 trimethyl mark via the chromodomain of its Eaf3 subunit. The H4 histone acetyltransferase (HAT) NuA4 does not, however, recognize trimethylated H3K36 despite sharing the

Eaf3 subunit. Bing Li (Stowers Institute, Kansas City, USA) addressed this interesting question of how yeast NuA4 and Rpd3S bind different histone-tail modifications despite both having the Eaf3 subunit. Using electrophoretic mobility-shift assays and domain-swap experiments, he showed that the combinatorial binding of the plant homeodomain (PHD) of the Rco1 subunit and the chromodomain of the Eaf3 subunit of Rpd3S determines its affinity and specificity for nucleosome substrates.

The H3K4 trimethyl mark is enriched at promoter nucleosomes of actively transcribed genes. H3K79 methylation is also associated with active genes, and plays a role in heterochromatin silencing. H3K4 trimethylation by the complex of proteins associated with the Set1 methylase (COMPASS) and methylation of H3K79 by Dot1 are dependent on monoubiquitination of H2B, which is directed by the Rad6/Bre1 complex; the mechanisms underlying these interdependencies are not known, however. Ali Shilatifard (Stowers Institute) used a biochemical approach to investigate the mechanism of such histone crosstalk. He has found that a COMPASS complex purified from a yeast *rad6* deletion strain had lost Cps35, the only subunit that is essential for viability. Shilatifard showed that this complex was defective in the di- and trimethylation of H3K4 *in vitro*, suggesting that the Rad6/Bre1 complex regulates the activity and stability of COMPASS. Surprisingly, he discovered that Cps35 also directly interacts with Dot1, and is required for H3K79 methylation by Dot1. This indicates that the Cps35 subunit of COMPASS translates the H2B monoubiquitination signal for COMPASS and Dot1 methylation of H3K4 and H3K79, although how the numerous other epigenetic marks communicate with one another remains to be determined.

Using a quantitative proteomics approach to identify readers of histone marks in human cells, Marc Timmers (University Medical Centre Utrecht, Utrecht, The Netherlands) showed that the general transcription factor TFIID reads the H3K4 trimethyl mark via the PHD domain of the TAF3 subunit. This interaction was stimulated by acetylation of H3K9 and H3K14 (typical of an active promoter), suggesting that H3K4 trimethylation may retain TFIID at active promoters. The extent to which the H3K4 trimethyl mark stabilizes TFIID-promoter interactions remains unclear.

Mechanisms for regulating Pol II

The rate of Pol II recruitment is generally regarded as a measure of the transcriptional output for a given promoter and the rate-limiting step in transcription. In *Drosophila*, however, notable exceptions include transcriptional regulation via pausing of bound Pol II (promoter-proximal pausing) in heat-shock genes and some proto-oncogenes. *S. cerevisiae* does not appear to regulate genes via Pol II pausing. Several laboratories have looked for evidence of paused Pol II throughout the *Drosophila* genome and found that it is more widespread than previously appreciated. Karen Adelman (National Institute of Environmental Health Sciences, Research Triangle Park, USA) has carried out a genome-wide search for proximally paused Pol II in *Drosophila* promoters by performing ChIP-chip with the Rpb3 subunit of Pol II, and found that around 1,000 genes were enriched with Pol II in the promoter but not throughout the coding region, consistent with the signature distribution for paused Pol II in flies. Additional techniques, such as permanganate footprinting, negative elongation factor (NELF) depletion, and ChIP-chip for the phosphorylated Ser2 residue of the Pol II carboxy-terminal domain, corroborated Pol II pausing. She found around 1,000 genes associated with stalled polymerase, including genes involved in development, reproduction, and responses to stimuli such as heat stress, oxidative stress, ionizing radiation, and the immune response. Adelman proposed that paused Pol II maintains a local chromatin architecture that is poised for regulated and rapid activation in response to stimuli. David Gilmour (Pennsylvania State University, University Park, USA) also conducted a genome-wide survey in *Drosophila* for paused Pol II, in this case by analyzing the distributions of two NELF subunits using ChIP-chip, and detected NELF in around 4,000 regions throughout the genome. Permanganate footprinting, which measures strand separation and is the most definitive assay for pausing, confirmed that more than 75% of the NELF-bound loci contained paused Pol II, leading Gilmour to estimate that at least 1,000 genes harbor a paused Pol II. Interestingly, the genomic location of these paused polymerases puts them abutting the +1 nucleosome, suggesting that the organization of *Drosophila* nucleosomes may contribute to pausing.

The presence of a paused Pol II implies that the cell uses specific mechanisms to release Pol II into a productive elongation-active state. John Lis (Cornell University, Ithaca, USA) concluded from cellular snapshot methods of protein crosslinking and DNA footprinting in *Drosophila* that changes in the chromatin structure at the heat-shock protein locus *HSP70* are dependent on binding of heat-shock factor (HSF), and precede Pol II movement into the coding region of the gene. He reported that inhibition of the activating transcription elongation factor kinase, P-TEFb, blocked the transition to elongation, suggesting that P-TEFb plays a critical role in regulating Pol II pausing. David Price (University of Iowa, Iowa City, USA) reported the use of various *in vitro* transcription systems derived from *Drosophila* to show that P-TEFb controls Pol II elongation status by regulating the ability of TFIIF and NELF to engage the elongation complex. He found that TFIIF alone was unable to associate with a Pol II complex containing NELF and the DRB sensitivity-inducing factor (DSIF). However, P-TEFb action shifted the balance, by blocking NELF retention and allowing TFIIF to bind to the productive elongation complex.

A recent genome-wide study in yeast found that Pol II was broadly detected throughout the genome, suggesting that more of the genome may be transcribed than previously thought. The strongest evidence for widespread transcription of most genes came from the distantly related fission yeast *Schizosaccharomyces pombe*. Brad Cairns (University of Utah, Salt Lake City, USA) has used whole-genome tiling arrays to detect small matched DNA-RNA hybrids, thus measuring the abundance of RNAs of all types. In addition to detecting sense transcripts at most genes, he found that many genes also produce antisense transcripts, and that islands of transcription exist within regions of heterochromatin.

From yeast to human, the eukaryotic cell meshes numerous levels of regulation to direct with exquisite precision transcriptional programs that dictate decisions on cell fate or respond to a rapidly changing environment. The emergence of high-resolution whole-genome nucleosomal maps coupled with the uncovering of histone crosstalk networks will provide deeper insight into long-standing transcriptional paradigms. Pol II is guided by many regulatory mechanisms during transcriptional initiation, pausing, and elongation, and thus understanding how the numerous Pol II-associated factors govern its transcriptional status will be an important focus of future studies. As distinctions between regulatory mechanisms dissolve, how the regulation of eukaryotic transcription is integrated in time and space will continue to captivate. We look forward with interest to next year's meeting.

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