Meeting report

Chromatin dynamics rule the genome

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Published: 31 October 2005

Genome ***Biology*** 2005, **6:**355 (doi:10.1186/gb-2005-6-11-355)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2005/6/11/355

A report on the FASEB Summer Research Conference 'Chromatin and Transcription', Snowmass, USA, 9-14 July 2005.

Changes in chromatin are important in the regulation of gene expression, and chromatin structure can be altered by nucleosome remodeling, modification of histone tails, or replacement of canonical histones by histone variants. A recent FASEB meeting on chromatin and transcription in Colorado covered a broad range of topics, including histone modifications, transcriptional regulation, histone variants, chromatin boundaries and higher-order structures. This report highlights just a few of the novel findings discussed at the meeting.

Histone modification and transcriptional regulation

Histones are subject to posttranslational covalent modifications that include acetylation, methylation, phosphorylation, ubiquitination, and ADP-ribosylation, which in combination form a 'histone code'. This code modulates transcription by affecting histone-DNA interactions or by creating sites that recruit other proteins. Tony Kouzarides (The Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK) described a novel cross-talk between proline isomerization and methylation of lysine on histone H3 that modulates transcription at certain promoters in the budding yeast *Saccharomyces cerevisiae*. When histone H3 proline 38 is isomerized by Fpr4, the yeast peptidyl-prolyl *cis-trans* isomerase, histone H3 lysine 36 methylation is suppressed. Kouzarides proposes that Fpr4 represses transcription by converting histone H3 proline 38 to its *trans* isoform, thereby preventing the histone methyltransferase Set2 from methylating lysine 36. Christopher Vakoc (University of Pennsylvania, Philadelphia, USA) reported that methylated lysine 9 of histone H3, a modification normally associated with heterochromatin, can also be found in the transcribed region of some active genes in mammalian chromatin.

Nicholas Laribee (University of North Carolina School of Medicine, Chapel Hill, USA) reported the identification of the cyclin Bur2, a substrate for the yeast cyclin-dependent kinase Bur1, as a regulator of both histone H3 lysine 4 trimethylation and the monoubiquitination of histone H2B (which involves the ubiquitin-conjugating enzyme Rad6). Both these histone modifications are associated with transcriptionally active genes. Synthetic lethal screens indicate a requirement for Bur1 kinase for transcriptional elongation, and Laribee reported that *BUR2* deletion mutants are unable to recruit the PAF1 complex to actively transcribing genes. PAF1 is a large protein complex that modulates RNA polymerase II function during transcriptional elongation. Thus, in addition to its role in histone tail modifications, Bur1 kinase plays a novel role in PAF1 recruitment and transcriptional elongation.

Replacement of the canonical histones H2A and H3 in nucleosomes by variant histones is another important mechanism by which the cell marks active or repressed regions of chromatin. Hiten Madhani (University of California, San Francisco, USA) described the use of chromatin immunoprecipitation (ChIP) in combination with genomic tiling microarrays to examine the distribution of the histone variant H2A.Z on *S. cerevisiae* chromosome III. These data revealed a striking trend: H2A.Z was present upstream of each gene, specifically in the promoter regions of both transcriptionally active and inactive genes in euchromatin.

In certain cases, transcription factors bind directly to histones. Ken Zaret (Fox Chase Cancer Center, Philadelphia,

USA) discussed an interesting mechanism whereby chromatin opening preceded histone modification early in liver development. This mechanism is mediated by Foxa transcription factors, which are necessary for the specification of endoderm-derived cells during embryonic development in mammals and were shown to bind histones independently of any chromatin modifications. Zaret reported that the DNA-binding domain and carboxyl terminus of Foxa1 is required for its histone-binding and chromatin-opening activities. In multipotent progenitor cells, rendering the chromatin accessible in this way allows covalent modification of histone tails and the binding of liver-specific transcription factors.

Histone modifications are important not only in transcriptional activation or repression, but also in marking nucleosomes in the coding regions of transcribed genes. Following the passage of RNA polymerase II during transcription, nucleosomes need to be carefully replaced. Fred Winston (Harvard Medical School, Boston, USA) discussed *S. cerevisiae* mutants lacking the protein Spt6, which interacts with RNA polymerase II and is important for transcriptional regulation. These *spt6* mutants show a marked increase in the use of cryptic promoters. Cryptic promoters are located in the coding region of genes such as *FLO8* and like conventional promoters, contain upstream activating sequences (UAS) and TATA box elements. In wild-type cells, specific nucleosome positioning mediated by Spt6 following the passage of RNA polymerase II may prevent transcription from these cryptic promoters. One of us (J.W.) discussed the role of the Rpd3 small histone deacetylase complex (Rpd3S) in repressing cryptic promoters. During transcriptional elongation, Set2 methylates histone H3 lysine 36. This signal results in Rpd3S histone deacetylation activity that represses transcription from cryptic promoters in the genes *STE11* and *FLO8*. These findings point to a hypothesis where histone H3 lysine 36 methylation provides a 'transcriptional memory' that allows nucleosomes to be properly replaced and deacetylated following passage of elongating RNA polymerase II.

Establishment and maintenance of chromatin domains

The establishment of regions of specific chromatin structure, known as chromatin domains, is important for telomere maintenance, gene silencing and the inactivation of entire chromosomes. Many presentations focused on how these domains are formed and established. Histone variants prove to be major players in establishing the boundaries between chromatin domains.

Karolin Luger (Colorado State University, Fort Collins, USA) discussed the alteration of chromatin structure by changes in nucleosome composition. Analysis of the structure of the macroH2A histone variant indicates that it prefers to form a hybrid nucleosome with H2A *in vitro*. The macro domain is able to interact with histone deacetylase complex 1 (HDAC1) and HDAC2. Thus, this variant may promote the formation of repressive chromatin domains through interaction with histone deacetylases. Steve Henikoff (Fred Hutchinson Cancer Center, Seattle, USA) has examined global distribution of the histone variant H3.3 in *Drosophila* S2 cells using genomic tiling microarrays. He reported that H3.3 was enriched at sites with high levels of RNA polymerase II and histone H3 lysine 4 methylation, indicating transcriptionally active genes, and that H3.3 correlated with histone H3 lysine 4 dimethylation. In transcribed genes, canonical histone H3 was gradually replaced by H3.3 both up- and downstream of the coding region with a sharp depletion of histone H3 at the promoter. Geneviève Almouzni (Curie Institute, Paris, France) compared mammalian histone variant H3.3 (tagged with the FLAG epitope for detection) purified from cytosolic, nucleoplasm and chromatin fractions. Soluble H3.3 in the nucleoplasm fraction lacks the active modifications (for example, histone H3 methylated at lysine 4), suggesting that these modifications occur on H3.3 after its incorporation into chromatin.

Covalent histone modifications also prove to be important for establishing chromatin boundaries. Vincenzo Pirrotta (Rutgers University, Piscataway, USA) discussed the role of Polycomb response elements (PREs) in establishing domains of histone methylation in *Drosophila* chromosomes. PREs are bound by the Polycomb (PcG) complex, which has histone-methylating activity. Genomic tiling microarray data indicate that histone H3 lysine 27 trimethylation is distributed throughout promoter and coding regions of the Hox gene *Ultrabithorax* (*Ubx*), but that the PcG complex is only present at the PRE. To explain the widespread methylation, a model has been proposed whereby the PRE interacts with DNA elements known as insulators, which are present at the boundaries between chromatin domains, and which block the spread of both gene silencing and methylation. Interaction between the *Ubx* PRE and the Su(Hw) insulator forms a DNA loop containing the *Ubx* gene, thus allowing the Polycomb group proteins bound to the PRE to methylate the entire gene. While PREs are not intrinsically able to cluster in the nucleus, many PREs are associated with insulator or boundary elements that promote both co-localization and functional interaction between PREs.

Genes present in heterochromatin are often silenced, and so it is important that the spread of heterochromatin from silenced regions into transcribed regions is prevented. Telomeres are used as one model to study the dynamic relationship between activating and silencing histone modifications. Maria Blasco (Spanish National Cancer Center, Madrid, Spain) showed that the heterochromatin domain at telomeres is important for maintaining telomere length, and that the retinoblastoma family of proteins is involved in maintaining the histone H4 lysine 20 trimethylation silencing mark on telomeric chromatin. Sharon Dent (MD Anderson Cancer Center, Houston, USA) has found that *GCN5* null

mice have shortened telomeres, implying that GCN5, a nuclear histone acetyltransferase (HAT), plays a role in telomere capping. Together, these findings suggest an interplay between *GCN5* HAT activity and histone methyltransferase activity in maintaining telomere length.

At the *HMR* mating-type locus in *S. cerevisiae* the expression of the **a** mating type is silenced by a mechanism involving spreading of heterochromatin. Rohinton Kamakaka (National Institute of Child Health and Human Development, Bethesda, USA) described a unique mechanism of barrier formation at this locus. At one side of the *HMR* locus a $tRNA^{Thr}$ gene maintains a nucleosome-free gap when bound by RNA polymerase III, and thus acts as a barrier to prevent spreading of the silenced HMR domain. This mechanism appears to work together with histone acetylation by the acetyltransferases SAGA or NuA4 to prevent the spread and binding of transcriptional repressors outside the *HMR* locus.

Nuclear organization of chromatin

The packaging and organization of chromatin in the nucleus was another common theme at the meeting. Frédéric Bantignies (Institute of Human Genetics, Montpellier, France) has examined long-range interactions involved in Polycomb-mediated gene-gene contacts. The *Drosophila* Hox genes *Abdominal-B* and *Antennapedia* are separated by 10 Mb, but they still interact specifically when both genes are repressed. Bantignies reported that this interaction depends on function of the PcG complex and the assembly of these regions of chromatin into PcG bodies which are the physical sites of PcG complex-mediated gene silencing. Thus, co-regulated genes may undergo nuclear compartmentalization.

Continuing with the theme of the influence of nuclear organization on transcriptional regulation, David Spector (Cold Spring Harbor Laboratory, USA) discussed a unique mechanism whereby RNA transcribed from a mammalian interferon-γ (IFN-γ)-responsive gene is retained in the nucleus until it is released by a cellular stress response. The CAT2 transcribed nuclear RNA (*CTN-RNA*) is an 8 kb RNA transcript with seven or eight IFN-γ-response elements. *CTN-RNA* is localized to micro-punctate regions called paraspeckles that are located in the nucleus. It is transcribed from the *mCAT2* gene through alternative promoter and poly(A) site usage and is the means of controlling the RNA level of its protein-coding partner, *mCAT2*. RNA editing is part of the mechanism responsible for the nuclear retention of *CTN-RNA*. When cells are treated with IFN-γ, *CTN-RNA* is cleaved to produce *mCAT2* RNA, which is then released from the nucleus. This rapid-response mechanism bypasses the need for new transcription and therefore speeds up the ability of the cell to produce a required protein product.

Although a fundamentally important question for the control of transcription, the organization of nucleosomes in higher-order structures has been unclear, but may now at last be resolved. Tim Richmond (ETH, Zürich, Switzerland) presented the crystal structure of a tetranucleosome to 9 Å resolution that showed a structure compatible with a two-start helix - two stacks of two nucleosomes with linker DNA running between them diagonally in a zig-zag pattern consistent with existing models.

From transcriptional control to nuclear organization, chromatin dynamics was presented at the meeting as a mechanism vital to proper genome regulation. Indeed, without very specific histone modifications or substitutions, the boundaries that organize chromatin in the nucleus would not be possible. It would seem, therefore, that chromatin dynamics rule the genome.