Epigenetics 7:10, 1211–1216; October 2012; © 2012 Landes Bioscience

Report on the 10th EMBL Conference on Transcription and Chromatin

August 25–28, 2012 EMBL Advanced Training Centre, Heidelberg, Germany

Sascha Duttke

Section of Molecular Biology; University of California, San Diego; La Jolla, CA USA

Keywords: EMBL, Heidelberg, chromatin, transcription, histone modifications, histone mimics, stem cells, pluripotency, reprogramming, RNA epigenetics, DNA hydroxymethylation, chromatin dynamics

Over 400 scientists from 38 countries gathered in the marvelous city of Heidelberg, Germany to attend the 10th EMBL Conference on Transcription and Chromatin. Surrounded by dark-green forest and overlooking a historic city, the charming environment of the EMBL as well as an all-star lineup of presenters attracted scientists from all over the world to discuss the latest findings and the future directions in the field. Superbly organized by Henk Stunnenberg, Eileen Furlong, Ali Shilatifard and Marc Timmers, the main topics of the conference included DNA, RNA and histone modifications, pluripotency and cellular reprogramming, transcriptional regulation, as well as chromatin dynamics and the effect of chromatin on gene regulation.

In a pleasant summer climate that facilitated a stimulating and collaborative atmosphere, over 50 outstanding talks and more than 200 posters were presented. I apologize in advance to those speakers whose excellent work I have been unable to include in this report due to space constraints.

Nucleic Acid Modifications, RNA Epigenetics and a Putative Sixth Base

Anjana Rao, from the La Jolla Institute of Allergy and Immunology (San Diego, CA), kicked off this very exciting session by introducing the potential mechanism of cytosine demethylation by the TET family enzymes and its role in cellular differentiation. TET proteins convert 5-methyl-cytosine (5-mC) to 5-hydroxymethyl-cytosine (5-hmC) and higher oxidized forms, 5-formyl-cytosine (5-fC) and 5-carboxyl-cytosine (5-caC), which can be reconverted to cytosine.[1-3] Genome-wide quantification of cytosine modifications in embryonic stem cells (ESCs) estimated 5% of all cytosines to be methylated and approximately 0.5% of them to be hydroxymethylated; the higher oxidized forms, 5-fC and 5-caC, are only present in a few thousand to a few hundred copies per ESC.[3] Dr Rao presented new insights into the mammalian TET protein family, which consists of three members. TET1 and TET2 are preferentially expressed in ESCs, while TET3 is mostly found in differentiated cells. TET2 lacks the functionally important CXXC domain, which got separated during evolution and now functions as an independent gene encoding IDAX (CXXC4). CXXC domains are intertwined double zinc fingers that bind unmethylated CG sequences in DNA. Dr Rao showed data suggesting that CXXC domains had an auto-inhibitory role in TET1 and TET3, and that IDAX had a dual regulatory role involving TET2 recruitment to DNA as well as TET2 degradation. IDAX was previously reported to be an inhibitor of Wnt signaling, therefore connecting Wnt signaling to DNA modification during embryonic development. Together, Dr Rao's work suggests the exciting model that TET protein function is regulated during differentiation and embryonic development, thereby changing cytosine modification patterns and the chromatin environment.

Dr Chuan He, from the University of Chicago, presented a technique to investigate the presence of 5-hmC on genome-wide scale. Protection of both 5-mC and 5-hmC from C to U conversion upon bisulfite treatment makes it impossible to distinguish between these two DNA modifications upon traditional bisulfite sequencing. This limitation can be overcome by TET-assisted bisulfite sequencing (TAB-Seq).[4] In short, in this method, the bacteriophage T4 β-glucosyltransferase selectively glycosylates the hydroxyl group of 5-hmC. Utilization of glucose with an azide group (N_3) allowed further modification such as addition of a biotin molecule, which facilitates enrichment. Glycosylated, and thus masked, 5-hmC is, unlike 5-mC, protected from TET-mediated oxidation. Hence, 5-hmC is read as C after bisulfite treatment while C and 5-mC are read as T. Using TAB-Seq, Dr He generated single base resolution maps of 5-hmC sites in the mammalian genome. 5-hmC displayed an asymmetric genome wide distribution, where 99% of all sites were at CG nucleotides. 5-hmC was further enriched in distal regulatory elements, including p300 and CTCF binding sites as well as DNaseI hypersensitive sites. Dr He also provided evidence for 5-hmC to be an active demethylation intermediate. It will thus be exciting to look forward to the functional roles of 5-hmC. TAB-Seq is commercially available from Wisegene.

Correspondence to: Sascha Duttke; Email: sduttke@ucsd.edu
Submitted: 09/06/12; Accepted: 09/06/12
http://dx.doi.org/10.4161/epi.22095

In the second part of his talk, Dr He presented FTO, an obesity-associated protein with oxidative demethylation activity. Intriguingly, FTO and homologs target the abundant mRNA modification N^6-methyladenosine (m^6A), converting it to adenosine.[5] Mammalian mRNAs carry in average three to six m^6A, often in the UTR region. The discovery of RNA demethylases shows, for the first time, the existence of reversible RNA methylation in mammalian cells, thus providing a novel mode of biological regulation.

Michiel Vermeulen (UMC Utrecht, Netherlands) utilized a combination of SILAC and DNA pull-down[6] to identify novel 5-mC or 5-hmC interacting or repelled proteins. Dr Vermeulen identified Klf4 as a novel methyl CG binding protein and showed that MeCP2 was also enriched among 5-hmC binding proteins. Proteins of the CXXC family were repelled by 5-mC and 5-hmC, while other proteins, such as RFX, bound 5-mC but not 5-hmC. His findings suggest that a large number of proteins specifically interact with the newly discovered cytosine modifications.

Pluripotency and Cell Reprogramming

Hendrik Marks, from Henk Stunnenberg's lab at the NCMLS at Radboud University Nijmegen (Netherlands), investigated the transcriptional and epigenomic foundations of ground state pluripotency. Mouse ESCs are traditionally cultured in media containing serum and the cytokine LIF. A few years ago, a defined serum-free ESC medium was developed that contains inhibitors of Mek and GSK3 kinases, known as "2i."[7] Cells cultured in 2i are reported to be more homogeneous in morphology and in the expression of various pluripotency factors. Dr Marks investigated the transcriptomes and epigenomes of ESCs derived and maintained in both types of media and tested the hypothesis that cells grown in 2i, as opposed to cells cultured in serum + LIF, are in a pluripotent ground state.[8] ESCs cultured in 2i showed lower expression of ectoderm and mesoderm linked genes and showed a 3-fold reduction in H3K27me3 at PRC2 target sites. Paused RNA polymerase II (RNAP II) was more prevalent in 2i ESCs. Notably, bisulfite genome-wide sequencing showed that DNA methylation was greatly reduced (mainly outside of CG islands) in 2i-cultured ESCs as compared with ESCs maintained in serum + LIF. The differences in transcriptome and epigenome between 2i-ESCs and ESCs grown in serum + LIF were reversible by changing the media type. Both cell populations, however, showed similar differentiation dynamics suggesting both culturing conditions yield equally potent cells. This suggests that there are at least two different pluripotent ESC states, with similar developmental potential.

Marc Timmers, from the University Medical Center Utrecht (Netherlands), showed that the canonical TFIID is a critical component of the transcription network of pluripotent mouse cells. Selective Taf knockdown revealed that most Tafs, but not Taf7 and 8, are essential to maintain the ESC state. Intriguingly, transient expression of Tafs greatly increased the reprogramming efficiency of fibroblasts by Oct4, Sox2, Klf4 and c-Myc (OSKM) factors. Together, these data reveal a central role for TFIID in cellular reprogramming.

Huck Hui Ng, from the Agency of Science, Technology and Research in Singapore, performed a siRNA library screen to investigate the transcription regulatory network required for Oct4 expression. With this screen, Dr Ng identified Prdm14.[9] In humans, Prdm14 associates with Ezh2 and is required for correct H3K27me3 levels. Prdm14-depleted cells were impaired in cellular reprogramming.

To understand better the limitations in the ability of transcription factors to engage chromatin and reprogram cells, Abdenour Soufi and Greg Donahue, from the lab of Ken Zaret (University of Pennsylvania), investigated the initial chromatin binding events during the conversion of somatic fibroblast cells to pluripotency with the Yamanaka factors OSKM. Dr Soufi showed that Oct4, Sox2 and Klf4 act as pioneers, engaging silent, DNase-resistant chromatin sites that lack known histone modifications, whereas c-Myc is dependent upon pioneer factors for binding such sites. Distal enhancer sites are engaged extensively prior to promoters, analogous to what has been seen in developmental fate choices.[10] The Zaret lab also discovered large domains of the somatic cell genome that are resistant to pioneer factor binding due to heterochromatic features and a specific histone modification. Diminishing the latter facilitates the binding and reprogramming by OSKM. These studies show how a mechanistic understanding of transcription factor engagement with chromatin reveals features by which cells stably maintain their differentiated states.

Tony Kouzarides, from the University of Cambridge (UK), showed that Padi4, the enzyme mediating citrullination of arginine,[11] also plays a role in pluripotency. Padi4 is expressed in ESCs. Proteomic analysis shows that most proteins contain one to two citrullinations, often at their active sites. Citrullinated proteins are involved in DNA repair, replication, transcription and chromatin remodeling. Overexpression of Padi4 induced activation of stem cell-specific factors like Tcl1 or Nanog, and Padi4 was shown to bind directly the promoters of these genes. Inhibition of Padi4 reduced the reprogramming capacity of neural stem cells. This raises the question of how Padi4 is involved in reprogramming. Dr Kouzarides identified a citrullination site in the DNA binding domain of the repressive histone H1. Citrullination of H1 likely evicts H1 from the chromatin, thus facilitating chromatin decondensation. Indeed, Padi4 was shown to control H1 levels on chromatin, particularly in promoter regions. Moreover, Padi4 was induced upon the reprogramming of cells to iPS cells. Histone H1, however, is not the only Padi4 substrate.

CTD Tyrosine Phosphorylation and RNAP II Regulation

Patrick Cramer (Gene Center, University of Munich, Germany) showed that CTD tyrosine phosphorylation is important to prevent the premature binding of RNAP II termination factors to phosphorylated serine 2 (Ser2P). Although Ser2P gradually increases during transcriptional elongation, Ser2P-binding termination factors are not recruited until transcription approaches the 3'end of the transcribed region. This is because transcribing RNAP II is phosphorylated at Tyr1 of the CTD, which stimulates

the binding of elongation factors but impairs the binding of termination factors. Phosphorylation of Tyr1 decreases before the polyadenylation site, which then facilitates binding of the termination factors.[12]

Histone Modification and Cryptic Transcription

Jerry Workman, from the Stowers Institute for Medical Research, presented his latest contributions to understanding Set2-mediated H3K36 methylation and its role in regulating histone exchange at transcribed genes. Set2 interacts with elongating RNAP II and methylates H3K36 co-transcriptionally. This not only prevents histone exchange over the coding regions, but also recruits the histone deacetylase Rpd3S, leading to suppression of cryptic transcripts. This suggested that co-transcriptional acetylation is a consequence of histone exchange. Probing for a mechanism to explain histone exchange suppression, Dr Workman's group found that the Isw1 and Chd1 chromatin remodelers associate with H3K36me3, to which the Isw1b complex is recruited. Catalytic mutants or depletion of ISW1 and CHD1 caused widespread cryptic transcription and caused increased exchange of histones at transcribed genes. Depletion of ISW1 predominantly affected infrequently transcribed genes, whereas CHD1 had a stronger effect on genes transcribed over ten times per hour. Depletion of both remodeling enzymes increased histone acetylation. Together, these data reveal an exciting new role for ISW1, CHD1 and chromatin structure in suppressing cryptic transcription in yeast.[13,14]

Stephen Buratowski (Harvard Medical School) presented a model for the role of co-transcriptional histone modifications and non-coding RNA (ncRNA) in gene expression. In collaboration with Lars Steinmetz (EMBL, Germany), Dr Buratowski found that Set3 suppresses the initiation of cryptic ncRNA transcription in regions where H3K4me2 is highest. Cryptic 3' end antisense ncRNAs overlap with the gene body and slow the kinetics of gene induction. Antisense transcripts likely function by recruiting H3K4me2 to the gene promoter. As H3K4me2 recruits Set3 and, thus, deacetylation,[15] cryptic transcription may function by delaying the gene regulatory circuit. This would ensure that gene expression is only upregulated by persistent signals.

Frank Holstege, from the University Medical Center Utrecht (Netherlands), presented his advances in the development of a method to analyze the effect that deletion of each regulator has on gene expression in yeast. This approach allowed the identification of linear signaling pathways and smaller protein (sub) complexes, as well as the topology of chromatin interaction pathways. Dr Holstege found that loss of H3K4 methylation by Set1 knockout caused derepression and delays in repression kinetics. Genes affected by Set1 showed H3K4me3 signatures at the 3' end, as well as 3' end antisense transcripts that are involved in gene repression. Set1 knockout decreased 3' end antisense transcription, thereby promoting sense transcription.[18] In yeast, H3K4me1–3 and Set1 are thus involved in repression through 3' end antisense transcription.

Expression Fine-Tuning

Transcription of genes in metazoans is often regulated during early elongation, when RNAP II pauses during initial RNA synthesis. Karen Adelman (NIEHS/NIH) reported that genes with paused RNAP II are particularly enriched in developmental or stimulus responsive pathways. The negative elongation factor (NELF) induces pausing to keep RNAP II near these promoters. Interestingly, Dr Adelman showed that NELF knockdown did not release RNAP II downstream, but decreased the level of RNAP II at the promoter and within the gene body, downregulating gene expression. This resulted from an increase in promoter nucleosome occupancy, suggesting that paused RNAP II and chromatin compete for promoter binding. Paused RNAP II was significantly enriched at genes encoding immune signaling components. In addition, NELF depletion globally dampened the immune response.[16,17] Pausing thus seems to target key factors in signal transduction and plays an important role in fine-tuning gene expression.

Chromatin Dynamics and Transcription

Shannon Lauberth, from Robert Roeder's lab at the Rockefeller University, presented a novel mechanism for DNA motif independent recruitment of the basal transcription machinery. Trimethylation of H3K4 selectively enhances p53-dependent transcription by stimulating the rate of pre-initiation complex (PIC) formation at the promoter. H3K4me3, but not H3K4, was sufficient to recruit the transcriptional machinery and initiate active transcription to an otherwise inactive promoter in vitro. Dr Lauberth found that the Taf3 subunit of TFIID was essential for PIC recruitment to H3K4m3. Genome-wide binding of Taf3 overlaps with H3K4me3, suggesting that H3K4me3 may contribute to global TFIID recruitment, potentially in a core promoter motif independent manner.

Modifications of histone tails are well known and were shown to play an important role in gene regulation. The role of modifications on the lateral surface of histones, however, is less known. Robert Schneider (Max Planck Institute of Immunobiology and Epigenetics, Germany, now at IGBMC in Strasbourg, France) presented a mechanism for a previously unidentified acetylation residue to function as a transcription stimulating, lateral surface chromatin mark. This residue is located close to the pseudo-dyad of the nucleosome and its modifications have the potential to impact chromatin dynamics and structure. Dr Schneider showed that acetylation of this residue correlates with gene expression, active chromatin marks and histone variants H3.3 and H2A.Z. It also co-localizes with H3K4me and marks active and tissue-specific enhancers. Transcriptional activity of chromatin templates was enhanced by acetylation of this single residue in vitro, which was rapidly acetylated by p300 upon gene induction in vivo. Mechanistic studies showed that acetylation on this core residue decreased nucleosome stability and increased the rate of DNA dissociation, likely stimulating transcription via histone

eviction. Dr Schneider's studies show that the list of discovered histone modifications is far from complete. He also highlighted the possibility of histones directly altering nucleosome structure through modifications of the nucleosomal core.

Histones are essential for viability. In an elegant genetic and biochemical approach, the groups of Jürg Müller (MPI of Biochemistry, Germany) and Alf Herzig (MPI of Biophysical Chemistry, Göttingen) further characterized the function of histones and their modifications in vivo. Using a conditional knockout of the histone cluster and a transgenic recovery system, they were able to investigate the importance of histones and their modifications and the role of histone variants in the fruit fly *Drosophila melanogaster*.[19] Due to its genetics, *Drosophila* is an excellent model organism for this study. Specifically, the developing wing disc offered a good system to analyze viability and division of cells with altered histones during development. With this assay system, Müller, Herzig and colleagues showed that histone variants are unable to compensate for the loss of S-phase synthesized core histones. However, despite showing somewhat reduced proliferation rates, loss of the linker histone H1 permitted cells to continue to proliferate possibly because the lack of H1 was compensated for by non-histone chromosomal proteins. This assay system also enabled the investigation of the importance of specific histone modifications. It was found that mutation of histone residues that are subject to particular modifications cause phenotypic alterations due to perturbed gene expression. Together, this study provided novel insights into the role of histones and their modifications during development.

Natural and Synthetic "Histone Mimics"

Alexander Tarakhovsky (Rockefeller University) discussed how pathogenic or synthetic proteins with histone-like sequences (histone mimics) can function as potent inhibitors of inflammatory gene expression. Histone mimics, such as the H3N2 influenza non-structural protein 1 (NS1) that is important for viral infection, can interfere with the human antiviral response via inhibiting the transcription elongation complex hPAF1C. This discovery also identified hPAF1 as a potential target for treatment in therapeutic applications. Upon viral infection, mammalian cells recruit chromatin remodeling complexes that open up the chromatin of antiviral gene promoters. This response specifically facilitates the expression of antiviral genes. Like the synthetic histone mimic I-BET,[20] the C-terminal tail of NS1 contains a sequence motif that mimics the N terminus of histone H3. The histone-like sequence (ARSK) can be methylated at lysine 229, by the histone modifying enzymes Set1C and Set7/9 or acetylated, by histone acetyltransferase TIP60. Biochemical analysis showed that NS1 interacts with the hPAF1C and the hCHD1 chromatin remodeling complex. This interaction may facilitate NS1 positioning in proximity to viral induced promoters. NS1 interferes with the transcription of virus-induced genes, while the transcription of housekeeping genes is not affected. Repression of virus-induced transcription of antiviral genes was hPAF1C-dependent. Moreover, depletion of the histone mimic of NS1 in recombinant H3N2 influenza greatly enhanced transcription of antiviral genes and reduced the viral infection titer. This suggests that the histone mimic in the tail of NS1 interferes with hPAF1C-dependent transcriptional elongation of antiviral response genes.[21]

Transcriptional Control by Nucleotide Biosynthetic Enzymes

Metabolic enzymes are usually thought to be downstream of regulatory networks. Peter Verrijzer (Erasmus University Medical Centre, Netherlands), however, showed that GMP synthetase (GMPS) can function as a gene specific transcriptional co-repressor.[22,23] GMPS is required for deubiquitination of histone H2B by USP7. Dr Verrijzer demonstrated that, in humans, GMPS stabilizes p53 by stimulating its deubiquitination by USP7. Knockdown of GMPS and USP7 affected the expression of p53 target genes. GMPS localizes mostly to the cytoplasm, where it maintains the pool of nucleotides. Upon stress induction, GMPS accumulates in the nucleus. However, depletion of the guanidine nucleotide pool causes a GMPS-mediated p53 response. GMPS retention in the cytoplasm is mediated by a ubiquitylation dependent mechanism, thus preventing p53 induction. When deubiquitinated, GMPS is imported to the nucleus and pairs with USP7 to stabilize p53.

Heterochromatin Regulation

Hypoacetylation and H3K9me enrichment are key features of heterochromatin. Yet, the requirements for establishing and maintaining a heterochromatic region are not well understood. Gunnar Schotta (LMU Munich, Germany) identified chromatin compaction by Suv4-20h2 to be essential for cohesin recruitment to heterochromatin.[24] Suv4-20h2 is stabilized at heterochromatin loci through multiple HP1 interactions. Cells with depleted Suv4-20h2 levels exhibits increased DNA accessibility and wider centromere distances. After a detailed analysis, Dr Schotta found that Suv4-20h2 condenses chromatin via cohesin recruitment, identifying a molecular cascade between H3K9me3/H4K20me3 and chromatin compaction.

Oliver Bell, from Gerald Crabtree's lab (HHMI and Stanford University), discussed the kinetics of H3K9me propagation and epigenetic inheritance. Dr Bell and colleagues found that direct targeting of HP1α could nucleate the formation of a heterochromatic H3K9me3 domain that propagated at rates of approximately 5.7 h per nucleosome in pluripotent cells and 6.9 h per nucleosome in mouse embryonic fibroblast cells. The researchers demonstrated that heterochromatic domains could be stably inherited through cell divisions even after removal of the HP1α stimulus. However, heterochromatin does not present a static barrier to transcription as strong activation could overcome HP1 dependent gene repression.[25]

Higher Order Chromatin Structure and Long Range Interactions

Wouter de Laat, from the Hubrecht Institute (Netherlands), presented a novel 3D analysis of the genome of pluripotent ESCs and showed that it undergoes major structural changes during

differentiation. Dr de Laat utilized 4C, a method that combines chromosome conformation capture with next-generation sequencing, to investigate all contact points on a particular sequence in the genome. The resolution of the method permitted mapping of regulatory sequences within a few kilobases and identified contact points as close as 10 kb.[26,27] Work from Dr de Laat and others showed that inactive chromatin regions show little contacts in ESCs, while the genome of differentiated cells is spatially more defined. Inactive regions in neural precursor cells showed more specific contacts than in ESCs. Reprogramming of neural precursor cells into iPS cells made the genome spatially less defined. These findings are consistent with those of Dixon et al.[28] and provide further evidence for the presence of spatially unorganized inactive chromatin in ESC. Dr de Laat also observed this phenomenon in more slowly dividing human iPS. What causes this change in chromatin architecture? Hi-C showed preferred contacts among Nanog binding sites in ESC but not in neural precursor cells, suggesting a role for pluripotency factors in shaping higher order chromatin structure.

Long Non-Coding RNAs and Transcript Regulation

Ramin Shiekhattar, from the Wistar Institute (USA), introduced the audience to a novel role of long non-coding RNAs (lncRNAs) in the regulation of gene expression. The human genome contains over 6,000 lncRNAs that range from 100 to 9,500 nt and average around 1,000 nt. The expression patterns of lncRNAs appear to be highly tissue-specific; in addition, the histone modifications H3K4me, H3K4me3 and H3K36me3 are often found at lncRNA genes. Long non-coding RNAs are not exported to the cytoplasm, and several lncRNAs were shown to activate their neighboring genes is cis.[29] These lncRNAs are mostly found to be associated with chromatin. Dr Shiekhattar found that lncRNAs could function in an enhancer-like fashion. This function is RNA dependent. For lncRNA-a7, he showed that Mediator confers this enhancer function. Mediator and lncRNA-a7 regulate DNA looping from the lncRNA promoter to the target gene promoter, thus activating the target gene in cis.

Outlook

In its 10th anniversary, the EMBL Transcription and Chromatin meeting celebrated a long-standing tradition of bringing together the leaders in the field and providing an interactive platform for discussion and socializing. Once again, this meeting impressed with an excellent line-up of speakers, a well-organized execution and a very pleasant environment. As a result, this only leaves us anxiously looking forward to the next meeting in 2014. On a final note, I would like to end this report by acknowledging the excellent job of Henk Stunnenberg, who, after organizing the last ten meetings, will leave the organizing committee; I anticipate that the remaining organizers, Eileen Furlong, Ali Shilatifard and Marc Timmers, will not disappoint in continuing with the tradition of making the EMBL Conference on Transcription and Chromatin meeting one of the best meetings in the field.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

I would like to thank Carolina Garcia Sabaté and Krasimira Todorova (EMBL Heidelberg) for administrative help, Epigenie (http://epigenie.com) for covering the conference registration fee, the UCSD Graduate School of Biological Sciences for travel support and Prof James T. Kadonaga for guidance, recommendations and continuous support. I am also grateful to all cited authors, Dr Barbara Rattner, Dr Alexandra Lusser, Dr Tamar Juven-Gershon and Dr Rebecca Wright for suggestions and editing of this manuscript. S.D. is supported by NIH grant GM041249 to James T. Kadonaga.

References

1. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009; 324:930-5; PMID:19372391; http://dx.doi.org/10.1126/science.1170116.
2. He Y-F, Li B-Z, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science 2011; 333:1303-7; PMID:21817016; http://dx.doi.org/10.1126/science.1210944.
3. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science 2011; 333:1300-3; PMID:21778364; http://dx.doi.org/10.1126/science.1210597.
4. Yu M, Hon GC, Szulwach KE, Song CX, Zhang L, Kim A, et al. Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome. Cell 2012; 149:1368-80; PMID:22608086; http://dx.doi.org/10.1016/j.cell.2012.04.027.
5. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011; 7:885-7; PMID:22002720; http://dx.doi.org/10.1038/nchembio.687.
6. Eberl HC, Mann M, Vermeulen M. Quantitative proteomics for epigenetics. Chembiochem 2011; 12:224-34; PMID:21243711; http://dx.doi.org/10.1002/cbic.201000429.
7. Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J, et al. The ground state of embryonic stem cell self-renewal. Nature 2008; 453:519-23; PMID:18497825; http://dx.doi.org/10.1038/nature06968.
8. Marks H, Kalkan T, Menafra R, Denissov S, Jones K, Hofemeister H, et al. The transcriptional and epigenomic foundations of ground state pluripotency. Cell 2012; 149:590-604; PMID:22541430; http://dx.doi.org/10.1016/j.cell.2012.03.026.
9. Chia NY, Chan YS, Feng B, Lu XY, Orlov YL, Moreau D, et al. A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. Nature 2010; 468:316-20; PMID:20953172; http://dx.doi.org/10.1038/nature09531.
10. Zaret KS, Carroll JS. Pioneer transcription factors: establishing competence for gene expression. Genes Dev 2011; 25:2227-41; PMID:22056668; http://dx.doi.org/10.1101/gad.176826.111.
11. Cuthbert GL, Daujat S, Snowden AW, Erdjument-Bromage H, Hagiwara T, Yamada M, et al. Histone deimination antagonizes arginine methylation. Cell 2004; 118:545-53; PMID:15339660; http://dx.doi.org/10.1016/j.cell.2004.08.020.
12. Mayer A, Heidemann M, Lidschreiber M, Schreieck A, Sun M, Hintermair C, et al. CTD tyrosine phosphorylation impairs termination factor recruitment to RNA polymerase II. Science 2012; 336:1723-5; PMID:22745433; http://dx.doi.org/10.1126/science.1219651.
13. Smolle M, Venkatesh S, Gogol MM, Li H, Zhang Y, Florens L, et al. Chromatin remodelers Isw1 and Chd1 maintain chromatin structure during transcription by preventing histone exchange. Nat Struct Mol Biol 2012; 19:884-92; PMID:22922743; http://dx.doi.org/10.1038/nsmb.2312.
14. Venkatesh S, Smolle M, Li H, Gogol MM, Saint M, Kumar S, et al. Set2 methylation of histone H3 lysine?36 suppresses histone exchange on transcribed genes. Nature 2012; 489:452-5; PMID:22914091; http://dx.doi.org/10.1038/nature11326.
15. Kim T, Buratowski S. Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5' transcribed regions. Cell 2009; 137:259-72; PMID:19379692; http://dx.doi.org/10.1016/j.cell.2009.02.045.
16. Gilchrist DA, Dos Santos G, Fargo DC, Xie B, Gao YA, Li LP, et al. Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. Cell 2010; 143:540-51; PMID:21074046; http://dx.doi.org/10.1016/j.cell.2010.10.004.

17. Gilchrist DA, Fromm G, dos Santos G, Pham LN, McDaniel IE, Burkholder A, et al. Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. Genes Dev 2012; 26:933-44; PMID:22549956; http://dx.doi.org/10.1101/gad.187781.112.
18. Margaritis T, Oreal V, Brabers N, Maestroni L, Vitaliano-Prunier A, Benschop J, et al. Two distinct repressive mechanisms for histone 3 lysine 4 methylation through promoting 3'-end antisense transcription. PLoS Genet 2012; In press.
19. Günesdogan U, Jäckle H, Herzig A. A genetic system to assess in vivo the functions of histones and histone modifications in higher eukaryotes. EMBO Rep 2010; 11:772-6; PMID:20814422; http://dx.doi.org/10.1038/embor.2010.124.
20. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, et al. Suppression of inflammation by a synthetic histone mimic. Nature 2010; 468:1119-23; PMID:21068722; http://dx.doi.org/10.1038/nature09589.
21. Marazzi I, Ho JSY, Kim J, Manicassamy B, Dewell S, Albrecht RA, et al. Suppression of the antiviral response by an influenza histone mimic. Nature 2012; 483:428-33; PMID:22419161; http://dx.doi.org/10.1038/nature10892.
22. van der Knaap JA, Kozhevnikova E, Langenberg K, Moshkin YM, Verrijzer CP. Biosynthetic enzyme GMP synthetase cooperates with ubiquitin-specific protease 7 in transcriptional regulation of ecdysteroid target genes. Mol Cell Biol 2010; 30:736-44; PMID:19995917; http://dx.doi.org/10.1128/MCB.01121-09.
23. van der Knaap JA, Kumar BRP, Moshkin YM, Langenberg K, Krijgsveld J, Heck AJR, et al. GMP synthetase stimulates histone H2B deubiquitylation by the epigenetic silencer USP7. Mol Cell 2005; 17:695-707; PMID:15749019; http://dx.doi.org/10.1016/j.molcel.2005.02.013.
24. Schotta G, Sengupta R, Kubicek S, Malin S, Kauer M, Callén E, et al. A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev 2008; 22:2048-61; PMID:18676810; http://dx.doi.org/10.1101/gad.476008.
25. Hathaway NA, Bell O, Hodges C, Miller EL, Neel DS, Crabtree GR. Dynamics and memory of heterochromatin in living cells. Cell 2012; 149:1447-60; PMID:22704655; http://dx.doi.org/10.1016/j.cell.2012.03.052.
26. de Wit E, de Laat W. A decade of 3C technologies: insights into nuclear organization. Genes Dev 2012; 26:11-24; PMID:22215806; http://dx.doi.org/10.1101/gad.179804.111.
27. Splinter E, de Laat W. The complex transcription regulatory landscape of our genome: control in three dimensions. EMBO J 2011; 30:4345-55; PMID:21952046; http://dx.doi.org/10.1038/emboj.2011.344.
28. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature 2012; 485:376-80; PMID:22495300; http://dx.doi.org/10.1038/nature11082.
29. Orom UA, Shiekhattar R. Noncoding RNAs and enhancers: complications of a long-distance relationship. Trends Genet 2011; 27:433-9; PMID:21831473; http://dx.doi.org/10.1016/j.tig.2011.06.009.