Epigenetics 7:4, 415-418; April 2012; © 2012 Landes Bioscience

Discussing epigenetics in Southern California

A report from the International Symposium on Epigenetic Control and Cellular Plasticity, UCI, December 15–16, 2011

Barbara P. Rattner

Landes Bioscience; San Diego, CA USA

Key words: epigenomics, epigenetics, DNA methylation, histone modification, sirtuins, cellular plasticity, chromatin, neuroscience

With the goal of discussing how epigenetic control and chromatin remodeling contribute to the various processes that lead to cellular plasticity and disease, this symposium marks the collaboration between the Institut National de la Santé et de la Recherche Médicale (INSERM) in France and the University of California, Irvine (UCI). Organized by Paolo Sassone-Corsi (UCI) and held at the Beckman Center of the National Academy of Sciences at the UCI campus December 15–16, 2011, this was the first of a series of international conferences on epigenetics dedicated to the scientific community in Southern California. The meeting also served as the official kick off for the newly formed Center for Epigenetics and Metabolism at the School of Medicine, UCI (http://cem.igb.uci.edu).

A Dynamic Epigenome

Because traditional methods only provide a static picture of the chromatin landscape, it is not surprising that Steve Henikoff (Fred Hutchinson Cancer Research Center, Seattle), who gave the first talk, chose to summarize efforts from his lab to develop newer methodologies that allow the study of a highly dynamic epigenome at single base pair resolution. He started by explaining that by combining the use of standard MNase digestion and paired-end sequencing to map the budding yeast epigenome, his group was able to characterize DNA-binding features of more than a hundred transcription factors, identifying protected and exposed regions around the binding site of each of them.[1] In another example, he explained that by using the "CATCH-IT" method for the study of the Drosophila genome, they showed that the rate of nucleosome turnover is faster at sites bound by Trithorax group proteins than at sites bound by Polycomb-group proteins;[2] a difference that is likely to be important for epigenome maintenance and gene regulation. Henikoff also studied the chromatin landscape before and after heat shock using S2 cells. These studies provided an extraordinarily detailed genome-wide view of RNAP II pausing and nucleosome turnover.[3]

Correspondence to: Barbara P. Rattner;
Email: barbara@landesbioscience.com
Submitted: 02/24/12; Accepted: 02/24/12
http://dx.doi.org/10.4161/epi.19815

Finally, he described a method that employs in vivo biotin labeling of nuclear envelope proteins present in individual cell types that allowed the purification of nuclei from different cell types in Arabidopsis for the analysis of cell-specific gene expression patterns and chromatin features.[4] Recently, the same method was used to isolate nuclei from muscle and non-muscle cells of adult *C. elegans*.[5] Henikoff and colleagues were able to identify genes that are specifically expressed in muscle tissues and found that these genes are depleted of nucleosomes at promoters and gene bodies in muscle cells but not in other tissues. This method could potentially be applied to compare epigenetic profiles among different cell types or tissues within any organism.

Also interested in developing assays that facilitate the study of a dynamic epigenome, Peter Jones's lab (University of Southern California, Los Angeles) used a single-molecule, high-resolution nucleosome positioning assay, called Nucleosome Occupancy Methylome-Sequencing (NOME-Seq),[6] to demonstrate that active (but not inactive) non-CpG island promoters display a nucleosome-depleted region (NDR), something previously studied only at CpG island promoters. Jones explained that the strength of the non-CpG NDR correlates with the expression level of the corresponding gene and suggested that epigenetic status of non-CpG island promoters should therefore be also taken into consideration in cancer studies. Jones explained that nucleosome occupancy precedes DNA methylation and that de novo DNA methylation does not occur in the absence of nucleosomes. Studying the process on individual DNA molecules, Jones and colleagues showed that the unmethylated OCT4 distal enhancer has a NDR that is maintained by binding of OCT4, which is itself required for OCT4 expression. Interestingly, de novo methylation follows the loss of the NDR, stabilizing a silenced configuration of the gene.[7] In a different example, Jones described his MYOD1 studies.[8] The MYOD1 gene is repressed by Polycomb-group proteins and is autoregulated (having binding sites for the protein it codes for in its enhancer and promoter). He observed that a NDR is characteristic of an expressing MYOD1 promoter. Exogenous MYOD1 activates its own transcription by binding first to the MYOD enhancer, which leads to a NDR at the promoter. Interestingly, cells that normally express MYOD1 (human rhabdomyosarcoma cell line) and cells in which MYOD1 is repressed (normal human fibroblast cell line) respond differently to forced expression of MYOD1 than cells in which MYOD1 is

silenced (RKO—colorectal cancer cell line). Jones found that the MYOD1 enhancers in both active and repressed MYOD1 cell lines (but not in RKO cells) are enriched in the H3K4me1 modification, which appears to mark a "permissive" state that is receptive and could potentially be activated by the binding of master regulatory factors. In genome-wide studies, Jones observed that a high percentage of Polycomb target genes have enhancers that acquire a permissive state, which could indicate a general mechanism for the regulation of cell-fate reprogramming.

When the Epigenome Says Silence

Moving the focus of attention now to heterochromatin function and formation, Hiten Madhani (University of California, San Francisco) discussed studies from his lab investigating how noncoding RNAs (ncRNAs) trigger heterochromatin formation. He presented data that identifies a conserved sequence-specific RNA binding protein that recognizes ncRNAs to promote silencing. This essential protein is physically recruited to centromeric repeat-derived ncRNAs in fission yeast and represents the molecular component linking ncRNAs and the histone H3 methyltransferase Clr4 in an RNAi-independent pathway. He discussed the implications of these findings as H3K9 methylation is generally triggered in fungi and animals, where it is clear that RNAi is not a universal trigger of heterochromatin formation.

Heterochromatin was also the focus of Thomas Jenuwein (Max-Plank Institute, Freiburg, Germany), who presented work from his lab on the identification of transcription factor binding sites within major satellite repeats. Specifically, his lab showed that Pax3 binds to satellite repeats and represses their transcription. In iMEF cells mutant for Pax3, he observed loss of heterochromatic histone methyl marks. In addition, double mutant cells in which Pax9 is also mutated, display massive deregulation of heterochromatic transcripts, indicating that Pax3 and Pax9 may perform redundant functions. Pax3 and Pax9 bind in the vicinity of the transcription start sites within major satellite repeats. Jenuwein proposed an interesting model in which the distinction between euchromatin and heterochromatin would be given by the way transcription factor binding sites are organized. In this model, heterochromatin is crowded with transcription factor binding sites, but these sites are not organized in the same way in which they appear in euchromatin (i.e., mainly in promoters and enhancers). He argued that the same transcription factors work in heterochromatin and euchromatin but, because in heterochromatin the binding sites lack the typical euchromatic organization, transcription factor binding is uncoordinated, defining that region to become heterochromatic (e.g., by triggering the recruitment of specific enzymes that modify chromatin).

Going (Epi)Genome-Wide

Identifying the epigenetic status of all genomic loci in each cell type at every given time is the goal of many laboratories working in the field. The potential application of this knowledge is enormous, including the design of diagnostic tools that could detect disease at earlier stages, just by analyzing the epigenome. A pioneer in this type of work, Bing Ren (University of California, San Diego) described efforts from his lab toward the identification of tissue-specific gene expression programs by genome-wide prediction of enhancers, promoters and insulators. A few years ago, his lab identified specific chromatin signatures present at different cis-regulatory regions.[9] Now, they have begun to systematically map these signatures in a genome-wide scale. Ren and colleagues performed the first survey of this kind using a ChIP-seq approach on 20 mouse tissues. Ren reported the identification of almost 300,000 regulatory sequences in the genome, the majority of which were confirmed by reporter assay arrays. These newly identified cis-elements provide annotation for 11% of the mouse genome, which represent a significantly larger portion than that occupied by protein-coding sequences. They could also associate putative enhancers with tissue-specific chromatin modifications and with the enrichment for lineage-specific transcription-binding motifs. Interestingly, out of 206 de novo motifs, 126 appear to match a known transcription binding motif and, of these, 75% have been shown to play a role in gene regulation in a particular tissue. Ren's lab is also trying to understand how these cis-regulatory elements are organized along the genome and work together in order to achieve gene regulation. Using Hi-C technology, which allows genome-wide analysis of higher order chromatin structure, he is identifying long-range chromatin interactions. This topological view indicates that the genome is composed of megabase sized topological domain structures that determine specific chromosomal territories, which confirm previous computational models.

Sirtuins Make an Appearance

Raul Mostoslavsky (Massachusetts General Hospital, Harvard Medical School, MA) and Katrin Chua (Stanford University, CA) moved the discussion toward sirtuins. Mostoslavsky and Chua talked about Sirt6, one of the seven mammalian sirtuins that shares homology with the yeast Sir2. Sirt6 is a chromatin-associated protein that promotes resistance to DNA damage and suppresses genomic instability in mouse cells. Loss of Sirt6 leads to an aging-like phenotype in mice that is likely to arise from its involvement in aging, metabolism and cancer.[10] In the absence of Sirt6, the uptake of glucose increases significantly. In Sirt6 knockout cells, lactate production increases and oxygen consumption decreases. Sirt6 regulates H3K9 acetylation at the promoter of glycolytic genes, causing an increase in their transcription.[11] Sirt6 therefore functions as a critical regulator of glucose uptake and glycolysis.[12] In his talk, Mostoslavsky discussed some new results indicating a role for SIRT6 in the modulation of transcription beyond its involvement in chromatin compaction. Furthermore, he presented new data suggesting a role for SIRT6 in cancer metabolism. Chua discussed ongoing work from her lab attempting to identify new cellular functions and contexts for Sirt6, in order to understand its role in physiology and disease. She explained that Sirt6 knockout mice die in approximately 4 weeks due to a hypoglycemic crisis but, when provided with a diet rich in fat, mice are able to survive past this crisis period. She showed that Sirt6 is not required for blood glucose maintenance

in adulthood. These longer-lived Sirt6 mice show behavioral defects that are reminiscent of mouse models of Alzheimer or autism, for example. These observations suggest a possible SIRT6 involvement in cognition and behavior.

Signaling to and from Chromatin

Paolo Sassone-Corsi (UCI) discussed how the cellular metabolism and the epigenome might communicate with each other in previously unsuspected ways. Specifically, he suggested that changes in the levels of cellular metabolites (which are partly controlled by the circadian clock) could influence the epigenome. Sassone-Corsi explained that because histone-modifying enzymes could "sense" the cellular metabolism, it is possible that they interpret the metabolic state of a cell at a given time by modifying chromatin in a specific cyclic manner.[13] As an example, the oscillatory nature of NAD^+ dictates oscillatory acetylation of Sirt1 targets, even though Sirt1 levels do not oscillate.[14] An important fraction of the genome is transcriptionally controlled in a circadian manner; it is intriguing to now start to understand how the circadian clock could act through the epigenome to exert its regulatory function.

Histone modifications signal the recruitment or activity of downstream effectors. The interpretation of this epigenetic signal is mediated by the so-called "readers," which are specialized proteins that are able to interpret the "epigenetic language." Or Gozani's lab (Stanford University) investigates the role of lysine methylation in disease. At this symposium he described a high-throughput peptide microarray assay designed to discover new "readers" containing chromatin-associated domains that could specifically recognize H4K20me2. Gozani and colleagues identified the BAH domain in Orc1 (the largest subunit of the origin recognition complex and previously implicated in primordial dwarfism) as the link between H4K20me2, Orc1 and primordial dwarfism. Binding of H4K20me2 appears to be required for efficient loading of ORC onto chromatin. Importantly, mutation in the BAH domain of Orc1 is implicated in the etiology of the Meier-Gorlin syndrome (a primordial dwarfism syndrome), due to the impairment of H4K20me2 recognition. There is therefore a potential role for H4K20me2 in determining organism size in mice. Remarkably, H4K20me2 depletion in zebrafish results in dwarfism.

RNA molecules are also important components of the dynamic epigenome. Axel Imhof (Ludwig-Maimilians Universität, München, Germany) discussed the role that RNA molecules play in chromatin. He explained that the binding of RNA to in vitro assembled chromatin appears to "open" chromatin, and the removal of RNA from chromatin leads to its compaction. This may be due to the removal of many (RNA-dependent) factors from chromatin, which he analyzed using an in vitro chromatin assembly system prepared from Drosophila embryos.[15] In his talk, Imhof suggested that the formation of a chromatin associated RNP network may be responsible for maintaining an accessible chromatin structure.

The role of cohesin in the regulation of gene expression was the topic of Kyoko Yokomori's (UCI) talk. In studies of the β-globin locus, she showed that cohesin, and the cohesin loading factor Nipbl, bind to the locus control region at the CTCF insulator region and distal enhancer, upon differentiation. Cohesin binding is critical for long-range chromatin interactions between the enhancer and the promoter and is important for β-globin gene expression in human cells. Nipbl haploinsufficiency affects cohesin binding, altering chromatin interactions and affecting gene expression.

Continuing the discussion on the mechanism by which the epigenome regulates transcriptional activation, Jean Marc Egly (IGBMC, INSERM, Strasbourg, France) talked about his studies of the intriguing role of nucleosome excision repair (NER) factors in transcription.[16] Egly explained that, upon gene activation, the RNAP II transcription machinery associates with NER factors at the promoter. Egly and colleagues observed that, in patients with silenced NER factors, the changes necessary for transcription to initiate (such as, H3K4me, H2K9me, H3K9/K14ac and DNA demethylation) do not occur. Deficiencies in NER factors impede the recruitment of other necessary remodeling factors that modify chromatin. Specifically, XPG and XPF appear to have a role in the formation of the necessary chromatin loop between the promoter and terminator.

Epigenetic Phenotypic Diversity

Joseph Ecker (HHMI and The Salk Institute for Biological Studies, San Diego) talked about his Arabidopsis studies of phenotypic diversity. His lab analyzed spontaneous changes in DNA methylation, which are able to produce stable epialleles that can modify the phenotype. Through examination of plants propagated by single-seed descent across 30 generations, Ecker and colleagues identified single methylation polymorphisms and CG differentially methylated regions that provide evidence for an epigenetic mechanism of phenotypic diversity.[17] These new epialleles are sequence-independent, can alter transcription and can be transmitted to the offspring, providing evidence for an epigenetic mechanism of phenotypic diversity that can occur in the absence of genetic mutation. Ecker ended his talk with the interesting question of whether these events could be regulated by the environment.

Epigenetics of the Brain

Jean Antoine Girault (INSERM, Paris, France) discussed the chromatin changes involved in the response to behavioral modifications. He explained that the dopamine signaling pathway is involved in several neurological and psychiatric disorders and in drug addiction. Dopamine regulates a nucleosomal response through a kinase/phosphatase cascade that mediates the nuclear accumulation of DARPP-32, a potent inhibitor of protein phosphatase-1. This accumulation causes phosphorylation of histone H3 on Ser10. Mutations in DARPP-32 alter the effect of drugs of abuse, which underlines the importance of DARPP-32 in this response.[18] Girault and colleagues are currently measuring transcriptional and epigenetic changes mediated by DARPP-32 using a series of clever tools that allow the isolation of polysomes or

nuclei from specific cells of interest. In preliminary results, they observed that cocaine exerts different effects on histone post-translational modifications in nuclei from dopamine receptor 1 (D1R)-expressing cells or in nuclei from cells expressing the dopamine receptor 2 (D2R).

Emiliana Borrelli (UCI) discussed the exciting possibility that neurological epigenetic effects could be induced by dysfunctional neurotransmitter's control of brain functions. She presented results from studies of dopamine receptor D2R conditional knockout mice in which D2R expression is lost specifically in presynaptic neurons. These animals appear to be excellent models of psychosis and schizophrenia, as suggested by their specific behaviors, which mimic the symptoms of these human neurological disorders. Analysis of RNA extracted from the frontal cortex, an area shown to be involved in schizophrenia, showed that almost 2,000 (out of a total of 25,000) RNAs were downregulated in D2R presynaptic mutant mice. Further study of this region showed that specific repressive histone marks are significantly enriched in these samples, compared with samples from wild type animals, suggesting that dopamine might mediate its effects in the frontal cortex via epigenetic events. As an example, Borrelli mentioned that the *Akt1* gene, which encodes one of the downregulated RNAs in D2R presynaptic mutants, shows significant enrichment of repressive histone marks at its promoter. It is appealing to speculate that disorders such as schizophrenia could be regulated by epigenetic events.

It is always fascinating to listen to researchers talking about their work. But, particularly at this meeting, their enthusiasm and vitality was refreshing and contagious. The arrangement of speakers gathered at this first Symposium on Epigenetic Control and Cellular Plasticity and the content of their presentations warrant success for many more international symposiums of this kind.

References

1. Henikoff JG, Belsky JA, Krassovsky K, MacAlpine DM, Henikoff S. Epigenome characterization at single base-pair resolution. Proc Natl Acad Sci USA 2011; 108:18318-23; PMID:22025700; http://dx.doi.org/10.1073/pnas.1110731108.
2. Deal RB, Henikoff JG, Henikoff S. Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science 2010; 328:1161-4; PMID:20508129; http://dx.doi.org/10.1126/science.1186777.
3. Teves SS, Henikoff S. Heat shock reduces stalled RNA polymerase II and nucleosome turnover genome-wide. Genes Dev 2011; 25:2387-97; PMID:22085965; http://dx.doi.org/10.1101/gad.177675.111.
4. Deal RB, Henikoff S. A simple method for gene expression and chromatin profiling of individual cell types within a tissue. Dev Cell 2010; 18:1030-40; PMID:20627084; http://dx.doi.org/10.1016/j.devcel.2010.05.013.
5. Steiner FA, Talbert PB, Kasinathan S, Deal RB, Henikoff S. Cell-type-specific nuclei purification from whole animals for genome-wide expression and chromatin profiling. Genome Res 2012; 22:766-77; PMID:22219512; http://dx.doi.org/10.1101/gr.131748.111.
6. Han H, Cortez CC, Yang X, Nichols PW, Jones PA, Liang G. DNA methylation directly silences genes with non-CpG island promoters and establishes a nucleosome occupied promoter. Hum Mol Genet 2011; 20:4299-310; PMID:21835883; http://dx.doi.org/10.1093/hmg/ddr356.
7. You JS, Kelly TK, De Carvalho DD, Taberlay PC, Liang G, Jones PA. OCT4 establishes and maintains nucleosome-depleted regions that provide additional layers of epigenetic regulation of its target genes. Proc Natl Acad Sci USA 2011; 108:14497-502; PMID:21844352; http://dx.doi.org/10.1073/pnas.1111309108.
8. Taberlay PC, Kelly TK, Liu CC, You JS, De Carvalho DD, Miranda TB, et al. Polycomb-repressed genes have permissive enhancers that initiate reprogramming. Cell 2011; 147:1283-94; PMID:22153073; http://dx.doi.org/10.1016/j.cell.2011.10.040.
9. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet 2007; 39:311-8; PMID:17277777; http://dx.doi.org/10.1038/ng1966.
10. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 2006; 124:315-29; PMID:16439206; http://dx.doi.org/10.1016/j.cell.2005.11.044.
11. Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, et al. The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. Cell 2010; 140:280-93; PMID:20141841; http://dx.doi.org/10.1016/j.cell.2009.12.041.
12. Zhong L, Mostoslavsky R. SIRT6: A master epigenetic gatekeeper of glucose metabolism. Transcription 2010; 1:17-21; PMID:21327158; http://dx.doi.org/10.4161/trns.1.1.12143.
13. Katada S, Imhof A, Sassone-Corsi P. Connecting threads: epigenetics and metabolism. Cell 2012; 148:24-8; PMID:22265398; http://dx.doi.org/10.1016/j.cell.2012.01.001.
14. Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P. Circadian control of the NAD$^+$ salvage pathway by CLOCK-SIRT1. Science 2009; 324:654-7; PMID:19286518; http://dx.doi.org/10.1126/science.1170803.
15. Scharf AN, Barth TK, Imhof A. Establishment of histone modifications after chromatin assembly. Nucleic Acids Res 2009; 37:5032-40; PMID:19541851; http://dx.doi.org/10.1093/nar/gkp518.
16. Le May N, Mota-Fernandes D, Vélez-Cruz R, Iltis I, Biard D, Egly JM. NER factors are recruited to active promoters and facilitate chromatin modification for transcription in the absence of exogenous genotoxic attack. Mol Cell 2010; 38:54-66; PMID:20385089; http://dx.doi.org/10.1016/j.molcel.2010.03.004.
17. Schmitz RJ, Schultz MD, Lewsey MG, O'Malley RC, Urich MA, Libiger O, et al. Transgenerational epigenetic instability is a source of novel methylation variants. Science 2011; 334:369-73; PMID:21921155; http://dx.doi.org/10.1126/science.1212959.
18. Stipanovich A, Valjent E, Matamales M, Nishi A, Ahn JH, Maroteaux M, et al. A phosphatase cascade by which rewarding stimuli control nucleosomal response. Nature 2008; 453:879-84; PMID:18496528; http://dx.doi.org/10.1038/nature06994.