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MINIREVIEW

Chromatin and Epigenetics at the Forefront: Finding Clues among Peaks

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The Keystone Symposium on Chromatin and Epigenetics, organized by Luciano Di Croce (Center for Genomic Regulation, Spain) and Yang Shi (Harvard Medical School, USA), took place 20 to 24 March 2016 at Whistler (British Columbia, Canada). The symposium brought together some of the most outstanding scientists studying how chromatin structure and epigenetic mechanisms regulate gene function in both development and disease. Junior scientists had the opportunity to interact with experienced researchers by presenting their work and discussing ideas and novel hypotheses. In order to foster interaction and networking, the scientific agenda was balanced with an extended social agenda. This meeting review describes several of the most provocative and exciting talks from the symposium, revealing how fast this research field is evolving and the profound impact it will have on human health.

The Canadian town of Whistler, famous as the largest ski resort in North America, held some of the sport events of the XXI Winter Olympics and Paralympic Games during 2010. This year, the so-called Athletes' Village hosted scientists who attended and participated in the Keystone Symposium on Chromatin and Epigenetics, which turned Whistler into a scientists' village for 5 days. The meeting's 320 participants included a large proportion of young scientists, with nearly 50% at the predoctoral or postdoctoral stage of their careers. Moreover, while a large proportion of participants (70%) had academic affiliations, the industrial sector was also well represented (with 12% of total affiliations), revealing the increasing interest of the sector in the epigenetics field.

The most relevant topics covered by this meeting are summarized in Fig. 1 with the relative levels of usage of keywords related to the chromatin and epigenetic research fields. Although cancer was the cellular dysfunction that occupied the greatest proportion of the meeting's interest, the impact of epigenetic research has extended to other functions, such as cellular differentiation and pluripotency, aging, and cellular memory. The ten-eleven translocation (Tet) enzymes were the most-studied protein family, although methyltransferases (MTs), in particular, the Polycomb group (PcG) of protein complexes, the mixed lineage leukemia (MLL) proteins, and the DNA methyltransferases (DNMTs), were in close proximity. DNA methylation seems still to be the most-studied epigenetic modification, although research findings concerning novel functions related to RNA modification were presented during the meeting. Recent advances in chromosome conformation capturing technologies (3C and 4C), together with clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 genome editing, provided new exciting insight into the functionality of regulatory elements, such as promoters and enhancers. Finally, novel methods for molecular analysis of chromatin were presented which will probably soon be part of the preferred techniques in the epigenetics research field, together with chromatin immunoprecipitation (ChIP) and massive parallel sequencing of the transcriptome (RNA-seq).

In this review, we highlight several of the most relevant findings presented during the meeting concerning the most discussed topics related to (i) the functionality of different epigenetic pathways, (ii) the enzymes involved in these pathways, (iii) new insight into epigenetic modifications, and, finally, (iv) new findings on nuclear organization.

FUNCTIONALITY

From single cells to multicellular organisms, cells have developed several epigenetic mechanisms to ensure the transmission of pathways resulting from experienced past stimuli to the next generation (1–3). The different epigenetic pathways are hierarchically placed at the functional level regulating transcriptional programs; therefore, their deregulation could profoundly impact the normal physiology of the cell and the progression of pathologies.

CANCER

Salvador Aznar-Benitah (Institute for Research in Biomedicine, Barcelona, Spain) discussed the power of chromatin organization to predict the regional accumulation of somatic mutations in cancer genomes. Correlative evidence indicates that genomic regions decorated with repressive chromatin marks show an increased propensity to accumulate somatic mutations (4). This mutagenic priming of heterochromatin regions suggests that modulating their levels could impact the tumor phenotype. In line with this, the Aznar-Benitah laboratory showed that epidermal tumor development is indeed affected upon modulating the levels of heterochromatin in a mouse model of skin carcinogenesis. However, heterochromatin modification not only resulted in significant changes in tumor burden but also affected genome instability and, interestingly, significantly altered the frequency of point muta-

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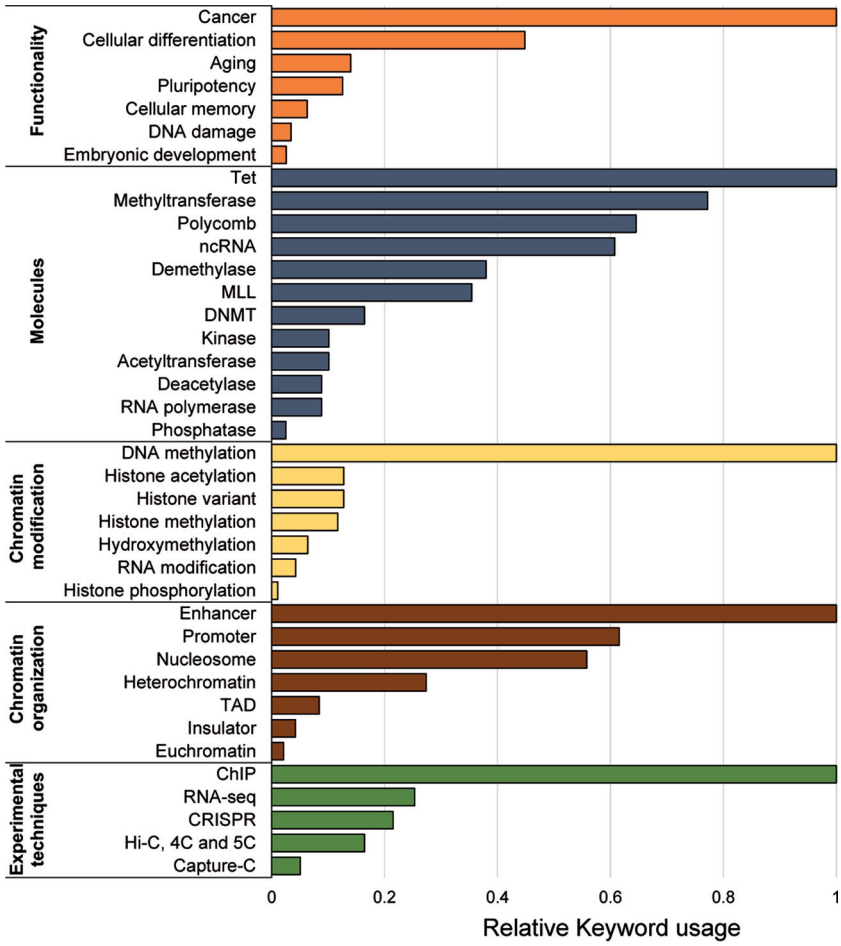

FIG 1 Relative levels of usage of keywords related to the chromatin and epigenetic research fields. The abstract book from the Keystone meeting was interrogated for the frequency of appearance of each selected keyword.

tions, suggesting that the link between chromatin and cancer is more complex than previously proposed (4, 5).

Or Gozani's (Stanford University, USA) research interests focus on understanding the molecular mechanisms of regulation of gene function through lysine methylation and how deregulation of lysine methyltransferases (KMTs) can contribute to cancer progression. He showed that the aberrant activity of two nonhistone KMTs promoted pancreas ductal adenocarcinoma (PDAC) progression (6, 7). He commented on published work showing that the cytoplasmic KMT SET and MYND domain-containing protein (SMYD3), which is overexpressed in several tumors, potentiates mitogen-activated protein (MAP) kinase signaling through MAP kinase kinase kinase 2 methylation, thereby promoting carcinogenesis (6). Additionally, Gozani showed functional and biochemical data supporting the idea of a role of SMYD2 during PDAC development and the identification of the stress response kinase (MAP kinase-assocated protein kinase 3) as a substrate, thereby pointing to SMYD2 as a regulator of the cellular response to stress in cancer cells (7). Globally, all his results point to SMYD3 and SMYD2 as excellent candidates for target therapeutics in cancer.

Recent associative studies have shown that up to 80% of diffuse intrinsic pontine gliomas (DIPGs), a form of pediatric high-grade gliomas, present the substitution lysine 27 to methionine (K27M) in histone H3 (8–10). Histone H3 K27 is a major target for the EZH2 methyltransferase, the enzymatic subunit of Polycomb repressive complex 2 (PRC2) (11). During the meeting, Danny Reinberg (New York University, USA) and Kristian Helin (Biotech Research and Innovation Center, Denmark) presented data on the molecular basis of DIPG etiology and pointed out the potential therapeutic agents for use against these gliomas. Reinberg shared the structural model of the human PRC2 complex, which provides insight into the propagation mechanism of trimethylated H3K27 (H3K27me3), whose function is to form the chromatin domain (12). The data support a model in which PRC2 activity relies first on stabilizing the EZH2 protein by trimerization with EED and SUZ12, followed by binding of the EED aromatic pocket to H3K27, leading to an allosteric activation of EZH2. Moreover, structure-based modeling and biochemical assays suggest that the K27M mutation binds to the SET catalytic domain of EZH2 and that PRC2 binds it with higher affinity than the wild-type histone. These observations indicating a correlation of the numbers of PRC2 molecules in different cells allow the proposal of a sequestration model. In line with this, Helin showed that H3K27me3 levels were globally reduced in a DIPG mouse model. However, some H3K27me3 high-content regions, including the Ink4-Arf

locus, were seemingly resistant to this reduction. Helin shared the idea that DIPGs cells might turn into PRC2-addictive cells during oncogenic transformation. Supporting this hypothesis, he showed that administration of EZH2 inhibitors led to an increase in life expectancy in mice xenografted with DIPG cells. Finally, Helin proposed a plan to use EZH2 inhibitors for this type of glioma and to monitor the Ink4-Arf locus activity as a biomarker for patient stratification and therapeutic engagement.

Other mutations in histone H3 have been found in other types of tumors, such as the K36M missense mutation in chondroblastoma tumors (13). Chao Lu (from the laboratory of David Allis, The Rockefeller University, USA) showed us that, similarly to H3K27M, the mutagenized version of H3K36M inhibits its targeting enzymes, the NSD1/2 and SETD2 methyltransferases. This results in a loss of H3K36me2/3 and a gain of H3K27me3 (14). This is thought to be the basis of transcriptional deregulation and tumorigenesis in this type of bone tumor.

In addition to mutations in the coding genome, researchers in the chromatin and epigenetic fields have recently expanded their interests to identify and characterize alterations in the noncoding regulatory elements as oncogenic drivers. The three-dimensional (3D) architecture of the eukaryotic genome is thought to contribute to gene regulation. Repressed and active regions are separated into different megabase-sized structural compartments, the so-called topologically associating domains (TADs), which show a high frequency of long-range genomic interactions within each TAD. The insulation of TADs is mediated by chromosome-structuring proteins, such as CTCF (15, 16). Global analysis of binding factor sequence showed that CTCF sites are among the sequences most frequently altered in human cancer and that these mutations occur frequently near cancer-associated genes (17). Abe Weintraub (from Richard Young's laboratory, MIT, USA) presented recent examples from T-cell acute lymphoblastic leukemia (T-ALL) in which recurrent microdeletions occurred at the boundary sites of insulated neighboring proto-oncogenes, which can drive cellular transformation (18). Bradley E. Bernstein (Harvard Medical School, USA) discussed recent results from studies of gliomas carrying oncogenic mutations in isocitrate dehydrogenase (IDH), showing altered genomic recruitment of CTCF and defects in TAD insulation in tumor-initiating cells. IDH gain-of-function mutants interfere with active DNA demethylation, thereby resulting in global hypermethylation that compromises the DNA binding ability of CTCF. Loss of CTCF binding results in a reorganization of the genome. In particular, IDH mutant cells overexpress the PDGFRα gene, a typical oncogenic driver of gliomas, due to aberrant contact with a normally insulated enhancer (19). These two presentations highlighted disruption of the 3D architecture of the genome as a driving force for oncogenesis.

Rab Prinjha (GlaxoSmithKline, United Kingdom) summarized the rapid recent progress in epigenetics drug development and the growing potential applications in human disease using brief examples from glycogen synthase kinase (GSK) of EZH2, histone deacetylase (HDAC), PAD4, and JMJD3 inhibitors. The emerging concept of epigenetic memory of disease and increasing understanding of its reversibility make these excellent mechanisms for drug development. In particular, Prinjha reported data on the potential efficacy of the GSK552 molecule, a specific inhibitor of lysine-specific demethylase 1A (LSD1), for the treatment of acute myeloid leukemia (AML) as well as small-cell lung carcinoma. In addition, he showed the potential impact beyond oncology of the modulation of epigenetic modifiers for the treatment of inflammatory diseases (20) using I-BET151 and I-BET762 as well as for the treatment of those induced by parasite infections (21).

In line with this, Saverio Minucci (European Institute of Oncology, University of Milan, Milan, Italy) presented a large set of *in vivo* data highlighting the benefit of using a combination of "epi-drugs" for treating AML. His results indicate that the presence of valproic acid, an HDAC inhibitor, effectively reduces the number of circulating leukemic cells and yet leaves the leukemia-initiating cells (LICs) untouched. In contrast, SAHA, a different type of HDAC inhibitor, can be used to effectively target LICs. Intriguingly, the combination of the two HDAC inhibitors has proven to be effective against both LICs and bulk leukemic cells, extending dramatically the survival of AML mice. Along this line, targeting the LSD1 histone demethylase has also proven to be highly effective, both *in vivo* and *in vitro*, and especially in combination with retinoic acid treatment.

CELLULAR MEMORY

Four relevant talks addressed the molecular mechanisms of cellular memory using different cellular systems. First, Keiko Ozato (National Institutes of Health, USA) showed results examining the implication of H3.3 deposition in interferon (IFN)-stimulated genes in mouse embryonic fibroblasts. At the chromatin level, a first IFN stimulation caused accumulation of H3.3 and H3K36 trimethylation at the promoter, gene body, and distal ends of induced genes. Functional analysis indicated that this accumulation is a mechanism to prime these genes for a rapid response after a second stimulation. She suggested that the innate immunity system developed this mechanism to speed and improve responses to external stimuli.

Second, Victor G. Corces (Emory University, USA) presented an extended molecular analysis of the mammalian sperm epigenome. He found that the set of genes that are activated in stem cells from the inner cell mass of the blastocyst are already bookmarked in the sperm by histone marks, RNA polymerase (RNAP) II binding, nucleosome distribution, and transcription factor and insulator occupancy. In line with this idea, Kiyomi Raye Kaneshiro (from Susan Strome's laboratory, University of California Santa Cruz, USA) presented results supporting the concept that PRC2 maintains gamete-inherited chromatin states through several embryonic divisions in worms. Mutants defective in pronuclear fusion retain inherited parental chromatin states in the unfused nuclei derived from the oocyte versus the sperm. In particular, in embryos resulting from the union of wild-type female oocytes and PRC2-defective male sperm, H3K27me3 is maintained only on chromosomes in oocyte-derived nuclei despite the presence of PRC2 in both parental nuclei. These two talks suggested that alterations in the already-bookmarked epigenome of parental germ cells can impact embryo development.

Finally, Giacomo Cavalli (Institute of Human Genetics, France) presented his recent results about transgenerational inheritance of epi-alleles mediated by PcG proteins in *Drosophila melanogaster*. He demonstrated that transitory contacts of PcG loci in the cellular nucleus can set up chromatin states and stabilize epigenetic gene silencing. He found that these epistates can be maintained for a large number of generations in flies, either under laboratory conditions or simulating wild-life conditions of flies in nature.

PLURIPOTENCY

The pluripotent state of embryonic stem cells (ESCs) is established by a unique protein network that interacts with largely open chromatin. This network is centered on pluripotency factors NANOG, OCT4, and SOX2, which control gene activity in a coordinated manner together with other transcription factors (TFs), such as estrogen-related receptor beta (ESRRB), and chromatin-modifying complexes, such as the PRC1 and PRC2 complexes (22). Ian Chambers (MRC, Edinburgh, United Kingdom) showed that the expression levels of some of these TFs can fluctuate in naive pluripotent cells. Interestingly, fluctuation of ESRRB expression marks the commitment of self-renewing ESCs to differentiation. He found that the members of a subclass of active enhancers are sensitive to ESRRB fluctuations in ESCs, thereby providing mechanistic insights into the classification of active enhancers in the naive pluripotent state.

Tian V. Tian (from Thomas Graf's laboratory, CRG, Spain) presented interesting results indicating that pluripotency exit requires, in addition to an initial disassembly of the pluripotency network, a concomitant activation of lineage-instructive transcription factors in ESCs. He showed that NSD2, independently of its methyltransferase activity, licenses enhancers of key mesodermal and endodermal transcription factors for their activation and that its knockdown causes a dramatic alteration of the pluripotency exit due to the failure to activate these mesoendodermal regulators.

Despite having a dispensable role during self-renewal, Polycomb complexes are required to engage appropriated differentiation programs (23). Recent work from Luciano Di Croce's group (CRG, Spain) has revealed the functionality of the different PRC1 subcomplexes during ESC differentiation (24–26). L. Di Croce presented recent unpublished data about the as-yet-unexplored modular composition of the PRC2 complex, showing data characterizing PRC2 as well as its C17orf96 protein component at the proteomic and genomic levels. He also discussed the functional role of the complex in establishing pluripotency during early mammalian development.

DNA DAMAGE

Three significant talks highlighted the link between epigenetics and DNA damage. In the first, Robert A. Martienssen (Cold Spring Harbor Laboratory, USA) showed that the selective K27 monomethylation of the H3.1 histone variant avoids overreplication of heterochromatin (27, 28). The H3.1 variants differ by only four and five residues in flowering plants and mammals, respectively. Martienssen's group discovered that the exclusive alanine 31 of H3.1 is "read" by arabidopsis trithorax-related protein 5 (ATXR5), which monomethylates K27 on H3.1 once it is loaded into newly replicated DNA. Martienssen provided data suggesting that small divergences in the sequence of H3 variants support the H3 variant-specific posttranslational modifications and that H3.3 enrichment on active regions may protect them from unscheduled H3K27me1 marking and gene repression.

During the second talk, Sandra Peiró (IMIM, Spain) discussed the functionality of the recently identified K4-oxidated histone H3 form (H3K4ox) (29). This H3 modification is carried out by the lysyl oxidase-like 2 protein (LOXL2). She has now found that H3K4ox is enriched in many tumor types with a poor prognosis, correlating with LOXL2 levels. Interestingly, she has identified a functional link between H3K4ox, chromatin compaction, and modulation of the DNA damage response pathway in triple-negative breast cancer cells. Using specific inhibitors of LOXL2, Peiró found increased sensitivity of cancer cells to DNA damage agents, therefore opening possibilities for novel therapeutic strategies for these aggressive forms of tumors.

Finally, John Whetstine (Massachusetts General Hospital, USA) has recently found that copy number variations (CNVs) in tumors are regulated by histone demethylase KDM4A (30). Low-oxygen exposure results in an increase in site-specific CNVs in cancer, as well as in primary cells, as a result of KDM4A stabilization. Whetstine showed that CNV gain is ameliorated by inhibiting KDM4A. In addition, he also presented data indicating that KDM4A expression is blocked by specific microRNAs (31). Their data show how genetic changes to epigenetic factors or processes modulating them can directly cause copy number alterations, revealing that copy number alterations are not random but rather can be controlled through epigenetic networks. Overall, J. Whetstine illustrated a novel mechanism that regulates genomic heterogeneity during oncogenesis.

ENZYMES

Tet enzymes. Despite its relatively recent discovery in 2009 (32, 33), the members of the ten-eleven translocation (Tet) family of proteins are among the top enzymes leading the ranking for the most recurrently named enzymes in the meeting (Fig. 1). Anjana Rao (La Jolla Institute for Allergy and Immunology, USA) discussed the newly discovered roles of Tet proteins in regulating promoter-enhancer interactions. In lymphocytes, her group found a strong correlation between 5-hydroxymethylcytosine (5hmC) abundance and chromatin accessibility.

A novel, unexpected role for Tet proteins and hydroxymethylation of RNA cytosines has been recently discovered by François Fuks (Free University of Brussels, Brussels, Belgium) (34). He provided a comprehensive transcriptional mapping of the RNA 5hmC in *Drosophila* cells, finding a particular enrichment in poly(A)-encoding RNAs. RNA 5hmC is highly abundant in *Drosophila* brain, and Tet-deficient flies show severe alterations in neural tissue formation together with a reduction of RNA 5hmC levels. Fuks has now extended his studies to mammals and presented data showing that RNA hydroxylation also occurs in mouse ESCs and in several adult tissues.

Yujiang G. Shi (Harvard Medical School, USA) suggested a molecular link between Tet proteins and glucose signaling. He identified Tet2 as a novel substrate of AMP-activated kinase (AMPK) that becomes phosphorylated at serine 99 upon starvation, thereby protecting it from degradation. In agreement with these findings, cells cultured in high-glucose media showed a reduced Tet2 half-life with no changes in transcription.

Polycomb group of proteins. One of the most controversial aspects of the functionality of PcG proteins is their mechanism of genomic recruitment. The initial studies in *Drosophila* reported evidence for specific DNA binding motifs that recruit PcGs to specific genomic regions (35). However, results of studies in mammals argue against a single mode of recruitment but rather point to several mechanisms of targeting PcGs to the genome, including interactions with sequence-specific binding proteins, histone modifications, noncoding RNAs (ncRNAs), and unmethylated DNA (11). Additionally, the classically accepted mode of recruitment, in which PRC2 binding to chromatin and H3K27me3 deposition precede PRC1 recruitment mediated by

the CBX subunits, has been recently challenged (36, 37). Mafalda Almeida (from Neil Brockdorff's laboratory, University of Oxford, United Kingdom) provided extensive data supporting a revision of the classical model for Polycomb recruitment in X chromosome inactivation (XCI). She used an elegant inducible model system for Xist gene expression to show that different PRC1 variants, defined by single PCGF subunits (PCGF1 to -6), all localize to Xist RNA domains. Fluorescence recovery after photobleaching (FRAP) analysis demonstrated that complexes with PCGF3 or -5 localized more stably and, accordingly, that knockout of these factors abolished recruitment of both PRC1 and PRC2 in response to Xist RNA expression. Besides identifying PRC1 variants required for XCI, her results support the proposal that noncanonical PRC1 activity precedes PRC2 recruitment.

In addition to the new C17orf96 PRC2 subunit reported by the Di Croce laboratory, another as-yet-uncharacterized polypeptide, C10orf12, is known to interact with the PRC2 core complex in a substoichiometric manner (38). Adrian P. Bracken (Trinity College Dublin, Ireland) has now identified C10orf12 as a PCL1 and PCL2 interactor by proteomics. This uncharacterized PRC2 interactor seems to directly bind to PRC2 in a mutually exclusive manner with C17orf96, JARID2, and AEBP2, all of which are substoichiometric components of PRC2. Bracken postulated the existence of several modular PRC2 complexes and that the appearance of C17orf96 and C10orf12 after mammalian radiation during evolution could have diversified the functionality of PRC2 complex in different subcomplexes.

EPIGENETIC MODIFICATIONS

DNA modification. During active demethylation, 5-methylcytosine (5mC) is transformed to 5hmC by Tet proteins. In order to complete the demethylation process, 5hmC is further oxidized to 5-formilcytosine (5fC) and then 5-carboxylcytosine (5caC). That last oxidized form is finally transformed into an unmodified cytosine by the action of the thymidine-DNA glycosylase (Tdg) and the base excision repair system (39). One of the major limitations that hamper studying the functionality of these DNA modifications in genome regulation is the lack of identified cognate binding molecules. Alex J. Ruthenburg (University of Chicago, USA) sought to resolve this by a biochemical fractionation of pig brain, combined with a pulldown assay using symmetrically modified oligonucleotides and mass spectrometry analysis. In this way, Ruthenburg identified WD repeat-containing protein 76 (WDR76) as a new 5hmC reader, hence uncovering a potential function of this protein in gene regulation.

During early mammalian development, cells in a selected group are instructed to become specialized primordial germ cells, with the fundamental role of propagating genetic and epigenetic information from parents to offspring. At this step, one of the most striking molecular events is the global genome demethylation of pluripotent epiblast cells differentiating into germ cells (40). The demethylation process is observed in both mouse and human primordial germ cells, although they differ substantially in their dynamics. While the demethylation process is completed in 5 days in mouse (40), this process is extended to 35 to 42 days in human (41–43). Wolf Reik (Babraham Institute, United Kingdom) shared data exploring the contribution of passive and active demethylation pathways to this process. Passive demethylation occurs by dilution of 5mC during DNA replication and the ineffective activity of DNA methylation maintenance enzyme DNMT1. In contrast, active demethylation is mediated by the action of Tet enzymes. Reik analyzed the contribution of each pathway to rapid demethylation in ESCs by shifting primed ESCs cultured under serum-plus-leukemia inhibitory factor (LIF) conditions to a naive state by moving them to media with Mek (mitogen-activated protein kinase and glycogen synthase kinase 3 [GSK3]) inhibitor-plus-LIF (2iLIF) culture conditions. Using a combinatorial knockout approach for all enzymes involved in both pathways, he determined that active demethylation is dispensable for the rapid demethylation that takes place when cells change from a primed to a naive state. Instead, DNMT1 and UHRF1 knockout cells accelerate the demethylation process drastically. Accordingly, the protein levels of UHRF1 and H3K9me2, required for UHRF1 binding at nascent DNA, are strongly reduced under naive conditions. Reik speculated that the process of DNA demethylation from the primed to the naive state is due to the dismantlement of 5mC maintenance machinery. He also postulated that the differential dynamics between DNA demethylation in mouse and in human primordial germ cells could be due to changes in the dynamics of UHRF1/DNMT1expression (48).

RNA modification. RNA can harbor nearly distinct 100 chemical modifications after its synthesis (44). Since their initial discovery more than 60 years ago, the vast majority of research on the modification of RNAs has focused on highly abundant RNA species, such as rRNAs, tRNAs, and snRNAs. However, the lack of sensitive methods has long hampered the characterization of RNA modifications of minimally expressed mRNAs (44). The possibility of the use of next-generation sequencing approaches has now increased the interest in RNA modifications of coding mRNAs and in their functions in normal and pathological situations. Recent research by Tony Kouzarides (Gurdon Institute, Cambridge, United Kingdom) has focused on identifying new roles for RNA modifications. A large screening for RNA enzymes involved in leukemia led to the identification of METTL3 and METTL14 as potential oncogenic candidates. Both enzymes catalyze the addition of a methyl group to the nitrogen in position 6 of adenosine (N^6-methyladenosine [m6A]). METTL3 and METTL14 bind to different sets of active genes, and METTL3 binds at the transcription start sites in the middle of the bimodal peaks of H3K4me3, where a CCAAT box is present. Specific knockdowns of these two proteins result in impaired growth of leukemic cells, pointing to them as new potential drug targets.

NUCLEAR ORGANIZATION

Promoter-enhancer interactions drive the initiation of transcription, and its dynamic association is thought to contribute to developmental gene patterning and diseases (45). Eileen Furlong (EMBL, Germany) summarized the presence and predictive value of six chromatin marks to distinguish active from inactive developmental enhancers, using characterized enhancers from *Drosophila* transgenic embryos as a training set (46). The researchers found that active enhancers have heterogenous levels of H3K27ac, H3K79me3, and RNA polymerase (Pol) II, which together are highly predictive of enhancers in an active state. Interestingly, H3K27ac is not present on all active enhancers, while H3K4me1 with H3K27me3 is indicative of enhancers in a repressed rather than poised state. In addition, she presented unpublished results showing the distribution of PcG proteins during *Drosophila* embryogenesis. She found that half of PcG-containing regions have features of Polycomb response elements (PREs) (H3K27me3 and

PRC1 components), are associated with minimally expressed genes, and have interesting regulatory properties.

In addition, Kouzarides proposed that promoter-enhancer loops are regulated by the members of a new family of long noncoding RNAs (lncRNAs), named topological anchor point RNAs (tapRNAs). Evidence includes the findings that (i) tapRNAs are present at points of chromatin looping, (ii) they are often present near developmental genes, and (iii) they regulate the expression of those genes.

Yang Shi reported the identification of a new chromatin complex containing two putative tumor suppressors, receptor for activated C-kinase 7 (RACK7) and histone demethylase protein KDM5C. This new complex binds a large number of active enhancers. Functional studies indicate that the complex works as a negative regulator of enhancer activity by controlling the switch between H3K4me1 and H3K4me3 (47).

Finally, Bing Ren (University of California, USA) presented the development of a CRISPR-Cas9-mediated genome-editing strategy to identify functional regulatory candidate regions. The strategy, called CREST-seq, was designed to discover and functionally characterize the *cis* regulatory elements within 2 Mbp of the human POU5F1 gene in human ESCs. He showed the identification of new regulatory elements on hPOU5F1 and, moreover, demonstrated the utility of CRISPR-Cas9-mediated genetic screening for functional annotation of noncoding DNA elements.

CONCLUSIONS

The latest progress of the field in several key areas was presented at the Keystone Symposium on Epigenetics and Chromatin. Moreover, beyond the specific questions addressed by the speakers and poster presenters, the meeting exposed the major open questions that remain unresolved: how do epigenetic mechanisms sustain gene expression programs? What are the epigenetic mechanisms that govern intergenerational gene expression programs? How can alterations in epienzymes drive cellular dysfunction, as occurs in cancer or aging? How do alterations in chromatin, beyond mutations in oncogenes and tumor suppressors, drive cancer progression? How do epigenetic writers, readers, and erasers exert their functions in gene regulation? What is the contribution of noncoding RNAs to chromatin architecture and gene regulation? What is the logic of chromatin compaction inside the nucleus? What is its contribution during gene regulation? Certainly, these questions will find answers in the upcoming symposia, and new open questions will face this relative young research field. We are sure that the interactions between experienced and young researchers fostered new ideas and provided the basis for challenging the old ones. Based on epigenetic mechanisms or not, the transmission of enthusiasm and knowledge was enhanced by these five fantastic days at Whistler.

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