Meeting report

Insights from Keystone: advances in the understanding of epigenetic regulation of the genome

Rebecca C Rancourt* and Nico Ruf†

Addresses: *Department of Physiology, Development and Neuroscience University of Cambridge, Cambridge, CB2 3EG, UK. †Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge, CB22 3AT, UK.

Correspondence: Rebecca C Rancourt. Email: rr315@cam.ac.uk

Published: 26 February 2009

Genome Medicine 2009, **1**:27 (doi:10.1186/gm27)

The electronic version of this article is the complete one and can be found online at http://genomemedicine.com/content/1/2/27

Abstract

A report on the Keystone Symposium on Epigenetics, Development and Human Disease, Breckenridge, Colorado, USA, 5-10 January, 2009.

At the Keystone symposium in Breckenridge over 200 participants gathered to explore epigenetic control of genome function through various model systems and biological processes. Topics touched on were chromatin dynamics, epigenetic mechanisms and regulation and environmental and disease influences on epigenetic states. Here we report some highlights of the meeting.

Chromatin dynamics

Bradley R Cairns (Huntsman Cancer Institute, Salt Lake City, USA) presented data showing that active DNA demethylation involves regulated coupling of deamination and base excision. Using a zebrafish demethylation assay system, he showed that activation-induced deaminase (AID) and methyl-CpG binding domain protein (MBD4) together cause genome-wide demethylation. Another factor that is involved in demethylation is Gadd45, which plays two roles: up-regulating transcription of the deaminase, as well as increasing interactions between AID and MBD4.

Steven Henikoff (Fred Hutchinson Cancer Research Center, Seattle, USA) spoke on histone variants and chromatin dynamics. Centromeric H3 has functional conservation between *Drosophila*, *Arabidopsis* and *Caenorhabditis elegans*, with no real sequence conservation. Phylogeny analysis showed a clustering of the H3 core (*Drosophila* and human 100% identical) and CenH3s as outliers. He spoke on investigating what possible structural regions are responsible for the differences in conservation.

Novel roles for core histone acetylation on H3K56 in longevity and tumorigenesis were described in a talk by Jessica K Tyler (University of Colorado Denver Health Sciences Center, USA). She summarized that the acetylation of H3K56 drives chromatin disassembly and reassembly at promoter regions, and chromatin assembly after DNA synthesis, and plays an important role in maintaining a normal life span in yeast.

Geneviève Almouzni (Institut Curie, Paris, France) gave an interesting talk on the challenges of DNA replication and repair and the roles of chromatin assembly factors in these processes. She presented recent data concerning a novel chaperone for the centromere protein-A (CENP-A) complex, which could be key in the maintenance and propagation of CENP-A at centromeres.

Petra Hajkova (Wellcome Trust Cancer Research, Cambridge, UK) gave a presentation on the mechanistic aspects of the genome-wide DNA demethylation in mouse germ cell development. Her data showed that the onset of DNA demethylation in primordial germ cells precedes chromatin remodeling, and provided evidence suggesting that the DNA repair process was linked to DNA demethylation.

Craig S Pikaard (Washington University, USA) outlined the subunit structure and functions of plant RNA polymerase IV and V. He revealed that the subunit composition suggests that RNA Pol IV and V evolved as specialized forms of RNA Pol II that have a role in production of noncoding transcripts for gene silencing and genome defense.

Functional organization of the nucleus

Jeannie T Lee's talk (Massachusetts General Hospital, USA) focused on X-chromosome inactivation (XCI), which in mammals exists in an imprinted and a random manner. She mainly focused on imprinted XCI and discussed her recent work on the timing and mechanism of paternal X-chromosome silencing during the transition from gamete to embryo. Her work suggested imprinted XCI takes place in distinct waves. One class of sequences on the paternal X is silenced first and its silencing may originate in meiotic sex chromosome inactivation (MSCI) in the paternal germline. This is then followed several divisions later by silencing of remaining genes and elements. The two classes of elements utilize different mechanisms and have differential requirements for the noncoding *Xist* RNA.

Gary Karpen (University of California, Berkeley, USA) talked about DNA repair in heterochromatin, which counts for about 30% of the *Drosophila* genome. Certain mechanisms, like homologous recombination by *RAD5*, seem to be suppressed in heterochromatic regions. He showed that initiation of DNA damage by X-ray irradiation disrupts the normally condensed heterochromatin and leads to the accumulation of RAD51 foci at the periphery of the heterochromatin in the nuclear periphery. He furthermore explained that depletion of Su(var)3-9, which is responsible for H3K9 methylation, in conjunction with DNA damage, results in activation of the G2 repair checkpoint and accumulation of RAD51 foci in heterochromatin. This suggests that the chromatin signature (for example, H3K9me) protects heterochromatin by excluding RAD51.

The role of the non-coding *Air* RNA gene in gene silencing was elucidated by Peter Fraser (Babraham Institute, Cambridge,UK). Air is required for silencing the paternal *Slc22a2* and *Slc22a3* alleles, which are specific to placenta. He showed that the *Air* transcript accumulates in the vicinity of the *Slc22a3* promoter and recruits G9a, which subsequently methylates H3K9. How the *Air* transcript targets the *Slc22a3* promoter is not known yet, but it results in stable silencing of the paternal *Slc22a3* by the heterochromatic mark H3K9me.

Long-range epigenetic control during development

Chromatin remodeling and erasure during germline specification in *C. elegans* were covered by William G Kelly (Emory University, Atlanta, USA). Like in mammals, an extensive chromatin remodeling occurs after *C. elegans* fertilization. Dr Kelly mainly discussed different linked events including H3K4me2 incorporation prior to zygotic genome activation and the maintenance of H3K4me marks, a process that seems to be independent of transcription in germ cells. In the germ cell lineage, there are two modes of transcriptional repression: a maternal PIE-1-dependent inhibition of pTEFb and mRNA production in the germline precursor P-cells, and a post-PIE-1 mechanism in the primordial germ cells (PGCs; PIE-1 degrades when the PGCs are born). This second mode of repression depends on a H3K36-specific HMTase, MES-4. Dr Kelly finds that MES-4 dependent H3K36 methylation is concentrated at promoters of *Hox* loci. In *MES-4* mutants he observes de-repression of transcription in the PGCs, ectopic expression of *Hox* genes in germ cells, and germ cell degeneration.

Vincenzo Pirotta (Rutgers University, New Jersey, USA) talked about his work on chromatin state at polycomb group (PcG) target genes in *Drosophila*. PcG proteins bind to certain polycomb response elements (PREs). In a genome-wide analysis he could show that PcG binding and H3K27me3 overlap. In contrast, repression of PcGs is neutralized by the members of the trithorax (TRX) family. A common feature of PcG target genes and their PREs in the active state is that they bind ASH1 and N-terminal but not C-terminal of TRX. UTX (ubiquitously transcribed tetratricopeptide repeat, X chromosome), a histone demethylase that was thought to be required for PcG gene transcription, is, in contrast, associated with all transcriptionally active sites.

Barbara J Meyer (University of California, Berkeley, USA) focused in her talk on the targeting of X-chromosome repression in *C. elegans*. In the worm, a specialized dosage compensation complex (DCC) binds to both X chromosomes in hermaphrodites to halve transcription. DCC recognizes its targets by *Rex*-sites, which contain a degenerated consensus sequence motif. The subsequent spreading of DCC is thought to occur through changes in chromosome structure.

Sam Schoenmakers (Erasmus MC University Medical Center, Rotterdam, Netherlands) provided a short presentation on MSCI in the chicken female germline. He explained that during chicken oogenesis, the heterologous Z and W chromosomes reach a state of complete synapsis that does not lead to an escape of MSCI. He showed that despite the pairing, the ZW chromosomes are transiently silenced from early pachytene to mid-diplotene stage. This silencing likely occurs by spreading of heterochromatin from W onto Z. His results show that MSCI occurs in birds with female heterogamety and suggest that there is an evolutionarily conserved mechanism that guides MSCI in the heterogametic sex.

Integrated epigenetic mechanisms

The role that RNA interference has in heterochromatin reprogramming in *Arabidopsis* and in fission yeast was

presented by Robert Martienssen (Cold Spring Harbor, USA). He showed that transposons are expressed in pollen but not the sperm cells of *Arabidopsis*. In pollen, loss of methylation and heterochromatin from transposons results in accumulation of mobile 21nt small RNA in sperm cells. In both yeast and plants, he proposed that RNA interference is required for spreading of H3K9me from repeats into genes via slicing of co-transcripts.

Steven E Jacobsen (HHMI/University of California, San Diego, USA) discussed some new factors that could play a role in the mechanisms of DNA methylation in *Arabidopsis*. He identified an *Arabidopsis* homolog of Spt5 named Asd, which is a downstream component specific to the DRM2 pathway. Asd plays a role in DNA methylation, as seen in asd mutants that have methylation defects, and cause methylation losses at other DRM-controlled loci. *In vivo*, he confirmed RNA involvement in that Asd interacts with AGO4 and also with NRPE1 (Pol V). He proposes that Asd is acting as an elongation factor with RNA Pol V.

Parental origin-specific epigenetic processes

Sundeep Kalantry (University of North Carolina, Chapel Hill, USA) gave a short talk on the requirement for *Xist* in the initiation of mouse imprinted X-chromosome inactivation. He showed analysis of X-linked gene expression in different stage pre-implantation embryos in *Xist* mutants that was generally the same as wild types, and suggested that imprinted X inactivation might be *Xist* independent. Normal silencing of most X-linked genes in two- and four-cell-stage embryos has not occurred. Silencing/monoallelic expression of X-linked genes began at the eight-cell stage with a high degree of silencing at the 16-cell stage.

Robert L Fischer (University of California, Berkeley, USA) provided a comprehensive analysis of the enzyme DEMETER (DME) as a DNA glycosylase domain protein. The DME family of proteins has co-opted the base excision repair pathway to demethylate DNA. DNA demethylation catalyzed by DME initiates imprinting in *Arabidopsis*. His data show that a functional DME maternal allele is required for plant reproduction. His studies suggest that DME is required for a global loss of DNA methylation in the endosperm, a tissue that supports the development of the embryo.

Wolf Reik's (Babraham Institute, UK) presentation covered the dynamics and mechanism of erasure of DNA methylation in the mouse germline. In the developing embryo, genome-wide erasure of DNA methylation occurs after the PGCs arrive in the genital ridge. It is proposed that PGCs at E13.5 of development have attained quite a low level of methylation. Genome-wide methylation data from different developmental stages were discussed, supporting the notion that the erasure might be an active process. He also reported that his lab is undertaking methylation analyses during reprogramming in mice mutant for cytosine deaminases, in order to examine if these enzymes are involved in erasure of methylation.

Nico Ruf (Babraham Institute, UK) presented evidence that transcription is required for establishment of maternal germline methylation marks at imprinted genes. By using the murine *Gnas* imprinted domain, he demonstrated that truncating the most upstream *Nesp* gene, which is transcribed through the whole locus in oocytes, including across the two germline differentially mehtylated regions (DMRs), disrupts methylation acquisition at the DMRs. He also showed that transcription through prospective DMRs in growing oocytes is common at other maternal DMRs. He therefore proposed that transcription is an essential component for the establishment of imprint marks in the female germline.

Environmental modulation of epigenetic states

Paul D Soloway (Cornell University, Ithaca, USA) spoke about two ongoing projects. Firstly, he is aiming to identify novel imprinted genes. He reported analyzing 200 murine candidate imprinted genes from a sequences-based prediction study that turned out to show biallelic expression. This encouraged him to develop a program that uses genetic and epigenetic characteristics to improve *in silico* imprinted gene prediction. A subset of genes that were predicted by the novel algorithm was tested in brain and placenta. Two genes turned out to be maternally expressed in placenta. In the second part, he introduced an innovative idea to test for several chromatin factors on a single molecule. He will be developing the technology in collaboration with others in the next years.

A talk about vernalization, an epigenetic phenomenon of environmental response, was presented by E Jean Finnegan (CSIRO Plant Industry, Australia). It is well established that many plants need cold periods to induce subsequent flowering. She reported about two potential factors - vernalization insensitive 3 (VIN3) and flowering locus C (FLC) - that regulate this response in *Arabidopsis*. *VIN3* gene expression increases as a consequence of cold but decreases afterwards. In contrast, *FLC* expression is depleted in the cold and remains low afterwards, associated with epigenetic changes at the *FLC* locus. She finally suggested that this is due to the replacement of H3.2 by H3.1. H3.1 can be marked with the repressive K27me mark, but H3.2 cannot. This exchange requires DNA replication and would provide a molecular explanation for the longstanding observation that only plants with actively dividing cells can respond to a vernalizing treatment.

In a brief presentation, Jay C Dunlap (Dartmouth Medical School, Hanover, USA) discussed the role of epigenetic mechanisms in the circadian rhythm in the fungal model

Neurospora. He showed that genes like *frq* (frequency gene) that are associated with the circadian clock undergo chromatin remodeling. Binding of CSW-1 and CHD2 to the *frq* locus is necessary for the remodeling that is associated with the daily cycle in activation of *frq* expression. FRQ negatively regulates its own expression by interacting with its activators, WC-1 and WC-2, which bind to the promoter. Binding of WC-2 is strongly cyclical, and CHD2 and CSW-1 are needed to sustain this rhythmic binding. DNA methylation of the *frq* promoter is also influenced by clock gene mutants, although the role of this modification remains obscure. After FRQ is expressed, it is gradually phosphorylated and eventually turned over in the proteasome, which allows the cycle, about a day in length, to start again.

Frédéric L Chédin (University of California, Davis, USA) presented data on DNMT3L (DNA (cytosine-5)-methyltransferase 3-like) interaction with multiple nucleosomal components to facilitate *de novo* methylation. It is well established that DNMT3L binds to the amino terminus of H3. By using histone peptide arrays, he identified H2A and H2B as additional binding partners. Interestingly, the identified binding sites are located side by side in the assembled nucleosome, implying that they correspond to one binding surface. A model was presented whereby interaction with the H2A/H2B sites anchors the DNMT3A/3L complex around its DNA substrate.

Developmental epigenetics

In an interesting talk by Matthias Merkenschlager (Imperial College London, UK), cohesin and its function in gene regulation and genome organization were discussed. Recent analysis has shown that cohesin is recruited to specific sites on mammalian chromosome arms by the insulator protein CTCF, and that, in turn, cohesin mediates CTCF's insulator function. New data indicate that CTCF and cohesin interact with the interferon gamma (*IFNG*) locus in a developmentally regulated fashion. Based on chromosome conformation capture assays, he proposed a model in which cohesin mediates long-range chromosomal interaction in *cis*, suggesting a novel function for cohesin in genome organization.

Gerald R Crabtree (Stanford University, USA) identified an embryonic stem (ES) cell specific chromatin remodeling complex, esBAF, essential for the pluripotent state. This complex is defined by a specific subunit composition and resembles the yeast SWI/SNF complex. Mice with null mutations in its specific subunits cannot produce ES cells, the inner cell mass, and indeed these subunit genes are haplo-insufficient for ES cell generation. Several of the subunits are known tumor suppressors. His group mapped the binding sites genome-wide and compared them to those genes regulated by the esBAF complex. Remarkably, all known pluripotency genes are targets of the esBAF complex. About 70% of the esBAF sites genome-wide co-bind with Oct4, Sox2 and Nanog. In addition to their role in the core circuitry of pluripotency, esBAF complexes bind to 84% of Stat3 and 75% of SMAD1 sites, but do not co-bind with polycomb complexes (consistent with recent information from several laboratories that polycomb is not required for pluripotency). The studies indicate that the genetically dominant role of esBAF in pluripotency arises from two mechanisms: first, from being an essential part of the core pluripotency transcriptional circuitry; and secondly, from being essential to mediate the actions of LIF-STAT3 signaling and bone morphogenetic protein-SMAD1 signaling, which are essential to prevent differentiation of ES cells.

Simon C Biddie (National Institutes of Health, USA) gave a brief presentation on nuclear receptor interactions with chromatin and the role of chromatin in nuclear receptor function. It was found that the glucocorticoid receptor can bind to DNaseI hypersensitive sites, which can be independent of (preprogrammed) or dependent on hormone (reprogrammed) using a quantitative PCR approach. By applying genome-wide sequencing, he found that glucocorticoid receptor binds invariably to sites of accessible chromatin in the genome. The phenomenon of receptor binding to accessible chromatin was shown to be true also for the aryl hydrocarbon receptor and to participate in cell-specific receptor binding. Chromatin accessibility was proposed to be a key principle for receptor binding and in mediating cell-specific binding events.

Qiaoning Guan (University of California, Berkeley, USA) gave a short talk on nutritional epigenetics in yeasts and human. Folate is a limiting factor for methylenetetrahydrofolate reductase (MTHFR) in maintaining *S*-adenosyl methionine levels. She and colleagues noted that folate deficiency leads to a decrease in histone methylation in yeasts. Mutations in the human *MTHFR* gene influence bulk histone methylation similarly to yeast.

Epigenetic mechanisms of disease

The role of epigenetics in neurobehavioral disorders, such as autism and schizophrenia, was outlined by Arthur L Beaudet (Baylor College of Medicine, Houston, USA). He demonstrated that genome-wide analysis using Angelman syndrome (AS) DNA methylation analysis and chromatin immunoprecipitation can detect epigenetic abnormalities in Prader-Willi syndrome (PWS) and AS postmortem brain. The probability of detecting epigenetic abnormalities in autistic and schizophrenic postmortem brains depends on the presence of abnormalities that are similar to those seen in PWS and AS.

Kristian Helin (University of Copenhagen, Denmark) highlighted the role of JMJD3 and H3K27me3 in regulating the expression of the *INK4A* and *ARF* tumor suppressor genes. JMJD3 is a member of the Jumonji demethylase family,

which is located close to tumor protein p53 (TP53) and is found expressed at lower levels in lymphomas compared to normal B cells. JMJD3 is required for proper induction of p16 in response to activated oncogenes. He showed H3K27me3 demethylation acts in the regulation of *INK4A*/ARF (alternative reading frame) locus, and that downregulation of JMJD3 is sufficient to immortalize primary mouse embryonic fibroblasts. These results show that *JMJD3* has properties of a tumor suppressor gene.

Andrew G Clark (Cornell University, Ithaca, USA) presented data from a transcriptome-wide screen for novel imprinted genes in neonatal mouse brain using short-read Illumina/Solexa sequencing. Novel imprinted genes were identified and he summarized the following trends: paternally expressed genes tend to be all or none, whereas maternally expressed genes tend to be partially biallelic, and neonatal mouse brain imprints tend to favor paternal expression. Dr Clark put forth the idea that individual interaction, and not necessarily sexual conflict, is what can drive the evolutional invasion of imprinting.

The symposium emphasized epigenetics as a key regulatory mechanism for the function of the genomes of various species. Overall, this meeting generated stimulating discussions and a deeper understanding in this field.

Abbreviations

AID, activation-induced deaminase; ARF, alternative reading frame; AS, Angelman syndrome; CENP-A, centromere protein A; DCC, dosage compensation complex; DME, DEMETER; DMR, differentially methylated region; DNMT3L, DNA (cytosine-5)-methyltransferase 3-like; ES, embryonic stem (cell); FLC, flowering locus C; *frq*, frequency gene; *JMJD3*, Jumonji domain containing 3, histone lysine demethylase; MBD4, methyl-CpG binding domain protein; MSCI, meiotic sex chromosome inactivation; MTHFR, methylenetetrahydrofolate reductase; PcG, polycomb group; PGC, primordial germ cell; PRE, polycomb response element; PWS, Prader-Willi syndrome; TRX, trithorax; UTX, ubiquitously transcribed tetratricopeptide repeat, X chromosome; VIN3, v*ernalization insensitive 3;* XCI, X-chromosome inactivation.

Acknowledgements

We are grateful to all speakers for their permission to include the presented data in this report. Also, we would like to thank Anne Ferguson-Smith and Steven Henikoff as well as the Keystone staff for organizing this inspiring meeting.