Meeting report

Epigenomes under scrutiny

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Published: 14 May 2008

Genome ***Biology*** 2008, **9:**308 (doi:10.1186/gb-2008-9-5-308)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2008/9/5/308

A report on the 3rd UK Stem Cell Meeting 'Epigenetics & Differentiation', London, UK, 11 March 2008.

Uncovering the foundations of the self-renewing capacity of stem cells and their ability to give rise to multiple cell types holds tremendous promise for understanding the basic principles of eukaryotic development, cell differentiation and genome reprogramming. Epigenetic mechanisms that affect chromatin organization play a fundamental role in these processes by controlling accessibility of the genome at the right time and right place. A recent stem-cell meeting in London provided a welcome opportunity to hear the latest advances on the fundamental determinants of stem-cell fates with an emphasis on epigenetic regulation in the mouse. The highlights selected here focus on the extent to which epigenetic mechanisms impact on stem-cell and developmental biology and on the underlying molecular machinery.

Epigenetic changes associated with the decision to differentiate

One challenge is the quest for chromatin signatures indicative of the developmental potential of a cell. This major task is being tackled by large-scale profiling of epigenetic modifications within the genomes of stem-cell populations that have broad developmental plasticity and the lineage-committed cells derived from them. Alexander Meissner (Harvard University, Boston, USA) presented a compelling technological advance enabling the analysis and comparison of global DNA methylation patterns in a high-throughput manner and, what is more, at nucleotide resolution. The power of this approach comes from bisulfite treatment of DNA, which converts all unmethylated cytosines to uracil, coupled with Solexa next-generation DNA sequencing technology. DNA fragments from, for example, restriction digests, are selected by size, generating a 'reduced representation' of the genome of a cell type or tissue. Libraries of bisulfite-converted DNA fragments can be compared to the same fraction of the genome across different samples, such as preparations from distinct developmental stages. Meissner and colleagues examined the dynamics of DNA methylation as mouse embryonic stem cells (ES cells) progress to neural precursors and neurons *in vitro*. Intriguingly, very little change was found in the overall distribution of DNA methylation between pluripotent cells and their differentiated derivatives. The alterations observed occurred primarily at CpG-poor promoters, which underwent dynamic methylation and demethylation. Similarly, distal regulatory regions of the transcription factors Olig1 and Olig2 became unmethylated upon their expression during cellular differentiation. This contrasts with CpG-rich promoters, most of which remain constitutively unmethylated throughout differentiation.

The histone proteins in chromatin are covalently modified at many sites, which affects chromatin activity and genome regulation. Meissner observed a strong correlation between levels of DNA and histone methylation under all developmental conditions analyzed and independent of the sequence context. Gain of tri-methylation of histone 3 at lysine 4 (H3K4me3), a mark indicative of active chromatin, correlated with DNA demethylation, whereas loss of H3K4me3 correlated with DNA methylation. Further dissection of the relationship between DNA methylation and different chromatin modifications during cellular differentiation should help to clarify how these marks integrate a wide range of signals that impact on chromatin function and how they contribute to the regulatory networks that underlie stem-cell fates. In this context, Angela Bithell (King's College London, UK) reported that neuronal stem cells and astrocytes differentiated from them have a common histone modification profile despite being transcriptionally distinct. She suggested that this is a reflection of the fact that astrocytes retain multipotency.

A paradigm for chromosome-wide epigenetic gene regulation during development is X-chromosome inactivation in female mammals. X inactivation involves the interplay between the *cis*-acting noncoding RNA *Xist*, changes in the

histone complement, and covalent modifications of DNA and histones. Bryan Turner (University of Birmingham, UK) described results consistent with a model whereby silencing of the X chromosome occurs progressively in differentiating ES cells, with different groups of genes becoming inactivated at different stages of differentiation. He proposed that this is linked to the configuration of the X-chromosome territory and the progressive spreading of *Xist* RNA through this territory. Turner also posed the question of how the male mammal survives with only one X chromosome, given that monosomy for autosomes is lethal in humans. Global expression profiling on the mouse X chromosome using microarrays provided a clue; in both males and females the expression of X-linked genes is increased about twofold on active X chromosomes relative to autosomes. Expression levels of X-linked and autosomal genes are thus balanced in mammalian genomes.

Neil Brockdorff (University of Oxford, UK) discussed mono-ubiquitylation of histone 2A at lysine 119, which is mediated by the Polycomb repressor complex 1 protein Ring1 and occurs on the inactive X chromosome as well as genome-wide. He reported that the RING-finger protein Mel-18 played a role in directing the repressor complex to nucleosomes. Interestingly, phosphorylation of Mel-18 is required for ubiquitylation of nucleosomes but does not affect the enzymatic activity *per se*: rather, it promotes recognition of the substrate. Brockdorff proposed that reversible protein phosphorylation of Polycomb complexes may regulate their binding and/or their activity, conceivably by causing conformational changes. Incorporation of ubiquitylated H2A into chromatin inhibits transcription in a reconstituted transcription assay *in vitro*, suggesting that this bulky modification on lysine 119 can affect transcription directly. This is in contrast to other histone modifications, which frequently function as protein-binding modules.

A fascinating question is which chromatin features have an impact on the first cell-fate decisions in the developing embryo. Previous work has implicated histone marks in this process. Maria Elena Torres-Padilla (IGBMC, Strasbourg, France) highlighted a critical role for monomethylation on lysine 20 of H4 during early mammalian development. She found that, in the mouse, the abundance of this histone mark increases after fertilization. Loss of the enzyme responsible, Pr-Set7, causes an arrest at the G2/M phase of the cell cycle and leads to pre-implantation lethality.

Reprogramming of the genome

Developmental plasticity lost during differentiation can be reacquired by resetting the appropriate gene-expression programs, a process that is critically dependent on epigenetic reprogramming. Jerome Jullien (University of Cambridge, UK) has developed a system to follow the steps of nuclear reprogramming to a pluripotent state in real time. He injected mammalian somatic nuclei into the germinal vesicles isolated from *Xenopus laevis* oocytes. An elegant reporter gene system in the somatic nuclei enabled him to detect reactivation of stem-cell genes as their transcripts contained a high-affinity binding site for a protein tagged with yellow fluorescent protein. Gene reactivation was associated with an of linker histone H1 mobility. Jullien showed that accumulation of the oocyte H1 in the transplanted nuclei is a critical step required for nuclear reprogramming.

Gain of totipotency, a characteristic of the germ line, involves genome-wide erasure of DNA methylation. To uncover the mechanisms that govern this reprogramming, Petra Hajkova (University of Cambridge, UK) investigated chromatin dynamics in developing mouse germ cells. She introduced the concept of two-step epigenetic reprogramming in the germ-cell lineage. Previous work had established that DNA demethylation occurs in primordial germ cells (PGCs) after they migrate to the gonads, at around embryonic day (E) 11.5. Hajkova observed distinct chromatin changes several days before DNA demethylation. Immunostaining of PGCs isolated around E8.5 revealed a signature pattern of histone modification reminiscent of pluripotency. These cells show loss of methylation on H3K9, enrichment of acetylation marks and tri-methylation of H3K27 as well as symmetrical methylation of arginine 3 on H2A and H4. The second step of remodeling occurs once the PGCs have reached the gonads and involves transient chromatin decondensation, alterations in the location of heterochromatin-associated proteins and in the nuclear architecture. Most striking was the extensive erasure of numerous histone modifications.

Hajkova argued that the mechanism for this is likely to involve large-scale eviction of histones from chromatin and histone replacement. In support of this attractive model she showed that chaperones implicated in histone exchange accumulate in PGCs during the second wave of reprogramming. Likewise, during this period an exchange of histone variants was evident, including the loss of H2AZ as well as H1. The onset of DNA demethylation precedes histone replacement at the second step of chromatin remodeling, suggesting that chromatin changes are largely the consequence of DNA demethylation rather than a prerequisite. This finding has exciting implications. Although it is known that this erasure of DNA methylation occurs in the absence of DNA replication and is apparently an active process, clarification of the mechanism and identification of candidate enzymes in mammals has remained a major challenge and a hotly debated topic. Results from plants have implicated the DNA repair pathway in DNA demethylation. Hajkova ended her presentation with the speculation that reprogramming in the mouse germ line may similarly entail a DNA-repair-driven demethylation mechanism, which in turn could induce chromatin changes and account for histone replacement. This will be an interesting avenue for future studies and may resolve a long-standing conundrum in the field.