Meeting report

Epigenetic regulation: DNA confers identity but is not enough to maintain it

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A report on the conference 'Epigenetics and the dynamic genome', 30 June-2 July 2005, Babraham, Cambridge, UK.

For a cell to remember its identity and its goal in life takes more than genetic information in the form of DNA. On and off states of genes have to be preserved, sometimes over generations. This is done by a set of mechanisms that are often called epigenetic, as they are not encoded by the genome. A meeting on 'Epigenetics and the dynamic genome' at the Babraham Institute in the countryside outside Cambridge was an opportunity to hear the latest progress in this fast-moving field.

New players in epigenetic regulation

Understanding the full spectrum of histone modifications and their effects on gene regulation is central to understanding epigenetics. Tony Kouzarides (University of Cambridge, UK) opened the meeting by revealing a new histone-modifying enzyme in budding yeast. The yeast protein Fpr4 can isomerize proline 38 in histone H3, which has the effect of inhibiting the methylation of lysine 36 on histone H3 (H3 K36). Yang Shi (Harvard Medical School, Boston, USA) updated us on lysine-specific histone demethylase 1 (LSD1), a histone H3 K4 demethylase. He reported that the cofactor for the repressor element 1 silencing transcription factor (coREST) binds LSD1 and is essential for its activity on nucleosomes. Genevieve Almouzni (Curie Institute, Paris, France) showed that the protein kinase complex Dbf4/Cdc7 phosphorylates the histone chaperone chromatin-assembly factor 1 (CAF1) during the early S phase of the cell cycle. Phosphorylation by Dbf4/Cdc7 stabilizes CAF1 in its monomeric form. This form binds proliferating cell nuclear antigen (PCNA), the replication sliding clamp, thus facilitating the role of CAF1 in replication-dependent chromatin assembly. Regulating CAF1 function is a novel way for the 'not so famous cell cycle kinase' Dbf4/Cdc7 to ensure a temporal coordination between DNA replication and nucleosome assembly.

Modifications to DNA itself are also crucial to epigenetic regulation. Researchers have been mystified by the molecular mechanisms responsible for the waves of rapid DNA demethylation that are essential for the early development of many species. Recently, various classes of DNA-modifying enzymes have started to emerge that could be responsible for this phenomenon. Primo Schär (University of Basel, Switzerland) showed that thymidine DNA glycosylase (TDG), unlike most DNA glycosylases, is essential for embryonic development. TDG removes the pyrimidines from G:T or G:U mismatches that occur by deamination of cytosine or 5-methyl cytosine, respectively. This potentially makes TDG part of a 5-methyl cytosine disassembly line, downstream of enzymes such as activation-induced cytidine deaminase (AID) that was reported by Svend Petersen-Mahrt (Cancer Research UK, Clare Hall Laboratories, South Mimms, UK) as being implicated in pluripotency in mammals. Schär showed that TDG-knockout cells have phenotypes implicating TDG not only in DNA repair but also in the transcriptional regulation of gene expression.

Genomic regulation by histone modification or histone replacement

One of the aims of epigenetics research is to determine the components that carry cellular 'memory' from cell generation to generation. Bryan Turner (University of Birmingham, UK) argued that as metaphase chromosomes are the inherited

entity during cell division, they are presumably the main source of somatic cellular memory. Using immunofluorescence studies on metaphase chromosomes, he showed that histone H3 isoforms mono-, di- and trimethylated at K4 show differing and characteristic distribution patterns. On the human X chromosome, these marks define a region rich in genes that escape X inactivation. Robert Feil (Institute of Molecular Genetics, Montpellier, France) showed that the H3 K9 methyltransferase G9A is essential for placenta-specific imprinting in the mouse. This fits well with his group's previous work that showed that histone modifications are associated with the maintenance of placental imprinting, whereas embryonic imprinting is dependent on DNA methylation.

As well as covalent modifications to histones and DNA, the behavior of chromatin can be modified by the replacement of the canonical histones with variant histones. Steve Henikoff (Fred Hutchinson Cancer Center, Seattle, USA) used chromatin-affinity purification of histone variant H3.3 in combination with tiling microarrays to determine the areas in the *Drosophila* genome that are enriched in this replacement histone, and compared the resulting profiles to published chromatin immunoprecipitation (ChIP) datasets for histone H3 dimethyl K4 and RNA polymerase II. In line with the presumed role for H3.3 in marking transcribed DNA, H3.3 abundance overlaps strongly with areas enriched in H3 dimethyl K4 and RNA polymerase II. Interestingly, H3.3 was enriched both upstream and downstream of transcription units, except for a strong dip in abundance over promoters that is attributable to nucleosome depletion over active promoters. Alain Verreault (Université de Montréal, Canada) presented an elegant study of a novel histone modification, H3 K56 acetylation, and its role in the repair of double-strand breaks in DNA. K56 is located at the DNA entry/exit point in the nucleosome and is in an acetylated state when histone H3 is deposited during S phase but is deacetylated thereafter. K56 acetylation persists near double-strand breaks until repair has occurred, however, suggesting a marking function. Indeed, a K56 to arginine substitution makes yeast very sensitive to agents such as bleomycin or camptothecin that induce double-strand breaks.

Genome reprogramming

The power of the environment over DNA is perhaps most evident in experiments where the identity of a cell or a nucleus dramatically changes as a result of alterations in the composition of the nucleoplasm. One of the classical systems for studying nuclear reprogramming has been nuclear transfer in *Xenopus*. An inspiring and thought-provoking talk by John Gurdon (University of Cambridge, UK), one of the pioneers in this field, started off a session on this topic. He showed that nuclei from both neuroectoderm and endoderm cells, taken from opposite sides of a blastula-stage embryo, can be efficiently reprogrammed (in 30% of cells) when transplanted into an enucleated *Xenopus* egg, yielding viable tadpoles. Analysis of lineage markers revealed, however, that 50-80% of the blastulae derived from these transplanted nuclei still express markers from the original program of their donor nuclei. This poses several questions. Are the markers evidence of a failure to completely erase the previous program, and if so, why does that not disrupt normal development? Is incomplete reprogramming the reason for the very low efficiency of cloning in mammals, and is coping better with aberrant gene expression the key to the much greater success of cloning in amphibians?

Rudolf Jaenisch (Whitehead Institute, Cambridge, USA) reported on the gene targets of the key transcription factors Oct4, Nanog and Sox2 in maintaining pluripotency in mammalian embryonic stem (ES) cells. ChIP data show that these factors share about 20% of their respective sets of target genes, including many Hox genes. Genes bound by all three factors can be active or repressed. Jaenisch suggested that an autoregulatory feedback loop between the *Oct4*, *Nanog* and *Sox2* genes and their products is important for maintaining pluripotency.

Over the past few years Austin Smith (University of Edinburgh, UK) has expanded our knowledge of the different signals that are important for maintaining cultured mouse ES cells in an undifferentiated state, or for forcing their differentiation. This has resulted in several protocols for controlled, quantitative differentiation of ES cells. He reported on his latest experiment, the derivation of neural stem cells from ES cells. ES cells can be differentiated into Sox1-expressing neural precursors. By addition of fibroblast growth factor and epidermal growth factor these precursors can be expanded into neural stem cells that no longer express Sox1 and that can be propagated indefinitely without losing their potential to differentiate into neurons, astrocytes or oligodendrocytes. They can also be transferred into a mouse brain, where they contribute to the appropriate lineages without causing tumors.

Chromosome dynamics

A paradigm for the regulation of gene expression on a chromosome-wide level is X-chromosome inactivation in female mammals. X inactivation is triggered by a noncoding RNA called *Xist* that spreads along the entire length of the X chromosome. Neil Brockdorff (MRC Clinical Sciences Centre, London, UK) addressed one question about the inactive X: how can repression from an initial point spread and be maintained chromosome-wide? To investigate this he is looking at X-autosome translocations, in which the spreading of X inactivation beyond the X-autosome breakpoint is often limited. Previous data suggested that repression can spread into the autosome but is not efficiently maintained through subsequent development, the so-called 'spread-and-retreat' model. Brockdorff's data provide an example of a different mechanism occurring in a specific X;autosome

translocation in the mouse. In this case, autosomal sequences resist the initial spreading of *Xist* RNA, and therefore of the inactivation signal. He discussed a hypothesis, first proposed by Mary Lyon a few years ago, that repression may occur more efficiently on the X chromosome as a result of its high density of LINE repeats. Interestingly, a large region of the autosome around the translocation breakpoint is depleted in LINE repeats, providing a possible explanation for the block in *Xist* RNA spreading. Phil Avner (Pasteur Institute, Paris, France) showed that in trophoblast stem cells the inactive X chromosome can switch into the active X, albeit at a low (10^{-5}) frequency. Using selection for activation of a marker on the inactive X, he reported that such cells are less stable in maintaining their X inactivation than are extraembryonic endoderm cells.

Recent years have seen a revolution in the understanding of the three-dimensional location of active or repressed genes in the nucleus, as a result of new chromosome conformation capture (3C) techniques or the optimization of older ones such as fluorescence *in situ* hybridization (FISH). Using three-dimensional FISH, Cameron Osborne (Babraham Institute, Cambridge, UK) showed that in the B cells of the immune system the *c-myc* gene is recruited to an RNA polymerase II focus upon transcriptional induction. The *c-myc* gene co-localizes in such foci with active immunoglobulin genes such as *IgH*, *Igκ* and *Igλ*, which are all situated on different chromosomes. Interestingly, the frequency of co-localization of *c-myc* and the different immunoglobulin genes correlates well with the frequency of different *c-myc*-immunoglobulin translocations, which cause lymphomas. Heidi Sutherland (MRC Human Genetics Unit, Edinburgh, UK) reported on the nuclear localization of ZFP647, a member of the family of Krüppel-associated box (KRAB) zinc finger proteins (ZFP) that has several hundred members in mammals but only one known in chicken and *Xenopus*. Transcriptional repression by KRAB proteins acts via KRAB-associated protein 1 (KAP1), which binds heterochromatin protein 1 (HP1). Sutherland showed that ZFP647 co-localizes with KAP1, HP1α and HP1β in nuclear foci upon differentiation of ES cells. Another KRAB protein, NT2, has a similar localization pattern. This may suggest that KRAB proteins repress genes by recruiting them to foci that may act as silencing factories.

Daniel Mertens (German Cancer Research Center, Heidelberg, Germany) investigates human cancer-associated genomic regions that show deletion of one allele without the other being mutated. He has found an example where, within tumor tissue, the genes in the non-mutated alleles are always silenced and late replicating. Silencing is not correlated with the paternal or maternal origin of the allele. Interestingly, treatment with the histone deacetylase inhibitor trichostatin A (TSA) or the DNA methylation inhibitor 5-azacytidine reactivated expression of the silent allele but did not affect the late replication timing. Dirk Schübeler (Friedrich Miescher Institute, Basel, Switzerland) presented the results of DNA immunoprecipitation with an anti-5-methylcytidine antibody and subsequent microarray analysis, covering the complete human genome as bacterial artifical chromosome (BAC) clones. Comparing the active and inactive X chromosome, he suggested that the inactive X is hypermethylated only in gene-rich regions but, unexpectedly, relatively hypomethylated in gene-poor regions. Also, gene-rich regions on autosomal chromosomes are more highly methylated than gene-poor regions, possibly to prevent aberrant gene transcription.

Rob Martienssen (Cold Spring Harbor Laboratory, New York, USA) wound up the meeting by updating us on the very fast-moving field of heterochromatin formation by RNA interference (RNAi). He showed that both transcription by RNA polymerase II (Pol II) and the mRNA-processing machinery are involved in RNAi-mediated silencing in the fission yeast *Schizosaccharomyces pombe*. A point mutation in the RNA Pol II subunit RPB2 abolishes the generation of small interfering RNAs (siRNAs) from centromeric transcripts. Deletion of Rik1, a subunit of the poly(A) polymerase complex, also leads to a loss of siRNA processing and loss of histone H3 K9 methylation. This fits well with the known recruitment by Rik1 of Clr4, the *S. pombe* H3 K9 methylase.

Nature has devised many complex and interlinked mechanisms to generate and maintain multiple cell types using the same genetic material. As it becomes increasingly clear that many diseases are due to defects that are not genetically encoded, understanding these mechanisms and to what extent they apply to disease is of utmost importance. Also, using these epigenetic mechanisms - RNA interference, for example - may sometimes be the only way to cure disease, as genetic manipulation is not always conceivable. This meeting succeeded in bringing together scientists from different disciplines in an attempt to forward our wider understanding of epigenetic phenomena, and it deserves a regular follow-up.