How epigenetics integrates nuclear functions

Workshop on Epigenetics and Chromatin: Transcriptional Regulation and Beyond

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The Juan March Foundation Workshop on Epigenetics and Chromatin: Transcriptional Regulation and Beyond was held in Madrid, Spain, between 7 and 9 February 2005, and was organized by M. Esteller, T. Kouzarides and V. Corces.

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changes, histone modifications and their effect on the transcriptional activity of the cell (summarized in Fig 1).

Chromatin: a two-sided structure with many faces

Chromatin constitutes the most fascinating structure in the cell. A eukaryotic cell must pack a huge linear molecule of DNA into a tiny compartment. The histone octamer, which comprises the protein core of the nucleosome, the repetitive subunit of chromatin, provides a smart solution as it facilitates the formation of a fibre that is able to compact DNA $\sim 10^4$ -fold.

Core histones can also be modified post-translationally in several ways, which in turn allows them to participate in regulatory mechanisms. In particular, their long amino-terminal ends, which span about one-third of their total length, protrude outside the nucleosome and interact with a myriad of nuclear factors. These factors read the signals encoded by post-translational modifications of the highly conserved Lys, Arg and Ser residues on the histones and participate in nucleosomal dynamics.

The duality of function of the nucleosome, as both a structural and regulatory subunit, also reflects the types of machinery with which it interacts: histone-modifying and chromatin-remodelling enzymes. Among the former, the most studied are histone acetyltransferases (HATs) and deacetylases (HDACs), which are involved in the balance of histone acetylation, and histone methyltransferases (HMTs), which catalyse methylation at Lys or Arg residues. Groups of ATP-dependent chromatin-remodelling activities regulate gene transcription by altering nucleosome positioning and structure. However, the boundaries between these two groups of machinery are fuzzy. For example, the recognition of chromatin-remodelling machineries is modulated by the recognition of modified amino-acid residues in the histones. Histone-modifying enzymes not only act on histone tails but also modify inner residues in the histone fold.

The role of histone modifications in the integration of all nuclear events was a theme present throughout the meeting, and the limits between the topics in each session were rather blurred. This reflects the fact that the interests of people working in transcriptional regulation, histone modifications, chromatin remodelling, signalling cascades and nuclear architecture are merging. To set the stage, the first session of the meeting was dedicated to the histone-code hypothesis.

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Introduction

In the past few years, there has been increased interest in the role of epigenetic modifications in transcriptional activity, as well as in integrating nuclear organization and function. As a result of these efforts, many histone-modifying enzymes, and the specific residues and contexts in which histone modifications take place, have been identified. The discovery that histone modifications are mechanistically linked at many levels with DNA methylation and nuclear organization has also led to the merging of fields that once worked independently of each other. This meeting provided a wonderful forum to discuss state-of-the-art findings on chromatin

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Fig 1 | Histone modifications and DNA methylation integrate nuclear function. Nucleosomal arrays in the context of chromosomal location and transcriptional activity are shown. Histone octamers are represented as grey circles. DNA is represented as a red line in which only methylated CpG dinucleotides are shown (red circles). Histone tails are lines protruding from octamers. A schematic representation of a chromosome is shown where different regions (satellite repeats, telomeric and subtelomeric regions and gene-rich regions) are indicated. Modified histone residues are indicated in each case. Although some modifications are general to active genes (acetylation and trimethylation at Lys 4 of H3), genes are regulated at many different levels including methylation of Arg 17 of H3, specific use of histone H3.3 variant and nucleosomal remodelling. The aberrant pattern of epigenetic modifications in cancer cells is also shown: promoter CpG islands of tumour-suppressor genes undergo DNA hypermethylation and loss of histone acetylation and trimethylation of H3–Lys 4, while repetitive sequences present DNA hypomethylation and loss of monoacetylation of H4–Lys 16 and trimethylation of H4–Lys 20.

A Rosetta stone for the epigenetic code?

The current model of how histone modifications are exploited by the cell, also known as the ‘histone-code hypothesis’, proposes that the combinatorial and/or sequential post-translational modification of histone residues can be read by nuclear factors to promote different processes and determine specific states (Strahl & Allis, 2000; Turner, 2000). However, we are still looking for a Rosetta stone to translate the hieroglyphic language of histone modifications. The specificity of the post-translational modifications of histones can be appreciated, for example, in the methylation of histone H3 at Lys 4 and Arg 17, which is closely linked to transcriptional competence, or the methylation of H3 at Lys 9 or H4 at Lys 20, which is associated with transcriptional repression (Turner, 2005; Peterson & Laniel, 2004). Similarly to the studies on gene function that showed the need for a detailed map of the genome, the future of epigenetics requires a detailed map of epigenetic modifications at a genomic scale. T. Jenuwein (Vienna,

Austria) discussed a study of the pattern of modifications in heterochromatin at different genomic locations, which further supports the view that a combination of epigenetic modifications is essential to explain the histone code. Although H3–Lys 9 and H4–Lys 20 trimethylation seems to be stable at several heterochromatic locations, significant variation is found depending on whether the cells are undifferentiated or differentiated embryonic stem cells, embryonic trophoblasts or fibroblasts (Martens *et al*, 2005). In this regard, retinoblastoma (Rb) deficiency has been related to the loss of H4–Lys 20 trimethylation at pericentric and telomeric chromatin (Gonzalo *et al*, 2005). Interestingly, the loss of monoacetylation at H4–Lys 16 and trimethylation at H4–Lys 20 in DNA repetitive sequences was revealed recently to be a common feature of malignant transformation (Fraga *et al*, 2005). Specific patterns of histone modifications and DNA methylation have also been found in imprinted genes, such as H3–Lys 9 trimethylation for the silenced allele. However, as W. Reik (Cambridge, UK)

showed, higher order structures could also be involved in imprinting, and the formation of chromatin loops could participate in the establishment of transcriptional domains (Murrell *et al*, 2004). This shows that the set of epigenetic modifications in a cell is specific to the cell or tissue type.

The complexity of the code depends not only on the presence of certain marks but also on the absence of others, by removal, occlusion and overwriting, as discussed by B. Turner (Birmingham, UK). The existence of crosstalk between histone modifications was also shown by the induction of H3–Lys 4 methylation on treatment with histone deacetylase inhibitors. Additional evidence of crosstalk was discussed by S. Berger (Philadelphia, PA, USA), whose group focuses on both the patterns and sequences of modifications and their correct timing. In yeast, histone H2B monoubiquitylation is associated with gene-associated H3–Lys 4 methylation and Lys 36 methylation (Henry *et al*, 2003). Berger also showed how H2B-deubiquitylating enzymes can have distinct genomic functions: the ubiquitin protease Ubp10 is involved in telomeric and gene-silencing functions, whereas Ubp8, the Spt-Ada-Gcn5 acetyltransferase (SAGA)-associated H2B deubiquitylase, is involved in gene activation (Emre *et al*, 2005). J. Workman (Kansas City, MO, USA) showed that the acetylation and deubiquitylation activities of the SAGA histone acetyltransferase complex are independent of one another. He also indicated that the expression of some genes, including arginosuccinate synthetase 1 (*ARG1*), is regulated by a balance of histone H2B ubiquitylation in the cell (Lee *et al*, 2005).

The hypothesis of the histone code obviously necessitates an understanding of how it is imposed and which machineries participate in its establishment. Histone acetylation and phosphorylation are easily reversed and thus can act as transient signals. Indeed, enzymatic activities that introduce and reverse such modifications are well characterized. For years, histone methylation has been considered a stable signal. Recently, the groups of T. Kouzarides (Cambridge, UK) and D. Allis (New York, NY, USA) reported an enzymatic activity that is able to demethylate arginine (Cuthbert *et al*, 2004; Wang *et al*, 2004). In the meeting, Kouzarides presented recent results on the H3–Arg 17 demethylation by peptidylarginine deiminase 4 (PADI4), which showed that this protein acts in concert with other factors known to be involved in transcriptional repression. A mechanism for histone lysine demethylation that involves the lysine-specific demethylase 1 (*LSD1*) gene has also been proposed (Shi *et al*, 2004).

A third issue that was discussed in the context of the histone-code hypothesis was the definition of the histone code itself. As B. Kingston (Boston, MA, USA) and Turner have pointed out, it may be necessary to include new elements, for instance post-translational modifications occurring in histone H1, or those taking place in other nuclear factors. Histone variants, such as H3.3, or RNA components or even the existence of particular structural features in the DNA could also be new elements to consider as part of a more general epigenetic code. For instance, S. Henikoff (Seattle, WA, USA) presented results on H3.3, a variant that is enriched in histone modifications associated with active chromatin, showing its colocalization with methylated H3–Lys 4 (McKittrick *et al*, 2004). The importance of non-coding RNA and the RNA interference (RNAi) machinery in *Polycomb* silencing in *Drosophila melanogaster* was discussed by V. Orlando (Naples, Italy), who showed how the RNAi machinery acts at Polycomb response elements to maintain homeotic gene silencing.

Conversely, CpG methylation, which is the most common epigenetic modification, has now fully entered the chromatin world after its connection to histone modifications was established (Bird & Wolffe, 1999). DNA methyltransferases (DNMTs) and methyl-CpG-binding domain (MBD) proteins are the main factors in establishing these connections. The fascinating interconnection between DNA methylation and histone modifications further shows that epigenetic modifications of a different nature compose a complex network. The recent discovery that DNA methylation alterations have an impact on both nuclear organization and histone modification patterns also supports this view (Espada *et al*, 2004). F. Azorin (Barcelona, Spain) showed how *Drosophila* dodeca-satellite protein 1 (DDP1) acts both by binding to single-stranded nucleic acids and by maintaining heterochromatin structure. Reik proposed that, during evolution, histone methylation might represent an older mechanism than DNA methylation in establishing gene imprinting. To support this hypothesis, Reik uses two main arguments: the imprinting of the distal chromosome 7 in the placenta, which depends on histone modification but not on DNA methylation, and the fact that the removal of DNMTs involves the loss of DNA methylation in imprinted genes but not a loss of imprinting (Lewis *et al*, 2004). He also suggested that autosomal imprinting and X-chromosome inactivation arose when the evolution of the placenta exerted selective pressure to imprint growth-related genes (Reik & Lewis, 2005).

Building the cell nucleus

The identification of nuclear factors that specifically bind modified amino-acid residues in histones provides the means to read these marks in order to regulate gene expression or other genome functions. The role of histone modifications in chromosomal and nuclear organization has also been determined recently, as described in several presentations at the meeting.

The functional compartmentalization of eukaryotic genomes is thought to be necessary for the proper regulation of gene expression. Chromatin insulators or boundary elements have been implicated in the establishment of this compartmentalization, as they may be involved in creating independent chromatin domains. V. Corces (Baltimore, MD, USA) discussed the dynamic nature of insulator organization. For instance, changes in insulator distribution that correlate with transcriptional activation can be observed during embryonic development. Identifying the proteins involved in the regulation of insulator activity is thus essential to understand these dynamics. In this regard, Corces showed how an insulator-interacting protein can act as a positive regulator of insulator activity by promoting the ubiquitylation of certain proteins and by inhibiting the sumoylation of others.

G. Felsenfeld (Bethesda, MD, USA) showed that barrier elements in insulators can also recruit histone-modifying activities, such as the histone methyltransferases SET7 and SET9 (specific for H3–Lys 4), protein arginine methyltransferases and histone acetyltransferases, to protect genes from the propagation of condensed chromatin structures. The consequence of insulator action on gene expression is the regulation of transcription by establishing and maintaining organization of the chromatin configuration. As Corces suggested, this concept may work in the nucleus using modules that function in a different manner during development.

Other molecules with a central role in chromatin integrity are chaperones, which promote chromatin rebuilding after disruptive events such as replication. It is clear that to maintain

epigenetic states fully, any change in the chromatin needs to be restored to maintain the tertiary structure. A specific architecture during replication ensures the maintenance of such three-dimensional organization for pericentric heterochromatin in mouse cells. One of the mechanisms participating in heterochromatin assembly is the interaction of heterochromatin protein 1 α (HP1 α) with the chaperone p150CAF1. The latter, which is targeted at replication foci, can thus coordinate histone deposition and provide a continuous supply of HP1 α proteins to these domains (Quivy *et al*, 2004).

Remodelling the chromatin

Chromatin has an essential role both in packaging the genome and in actively regulating its function at specific regions. This dual role is modulated by a wide variety of enzymes that covalently modify histones and/or DNA or that affect nucleosome stability by disrupting histone–DNA contacts. Although the biochemical properties of most chromatin-modifying enzymes have been well characterized, and the links between histone- and DNA-modifying enzymes and ATP-dependent chromatin-remodelling complexes have been established, the importance of their concerted action has only just begun to emerge. Chromatin remodellers may use different mechanisms to exert their function, including histone dimer removal or exchange, sliding and looping. At the meeting, Kingston presented restriction enzyme accessibility data that support a mechanism involving loop formation in mating-type switching/sucrose non-fermenting (SWI/SNF) remodelling complexes, which are involved in gene activation. The activity of chromatin remodellers appears to be gene specific. M. Beato (Barcelona, Spain) showed that the outcome of nucleosome remodelling by SWI/SNF depends on the specific DNA sequence in an inducible promoter (Vicent *et al*, 2004). Another example to illustrate this combination of chromatin-modifying activities was provided by F. Rauscher (Philadelphia, PA, USA), who showed that association of the Krab-associated protein 1 (KAP1) to euchromatic promoters provokes a repressive state that involves the recruitment of the Mi2 nucleosome-remodelling complex, HP1 and the SET domain bifurcated 1 (SETDB1) histone methyltransferase.

K. Struhl (Boston, MA, USA) presented data on how the opening of the chromatin can also be associated with structural phenomena, such as the exclusion of histones from the transcriptional start point of many genes in yeast. He also discussed how a reduced rate of RNA polymerase (Pol) II elongation may lead to premature dissociation along the chromatin template (Mason & Struhl, 2005).

Environment and epigenetic modifications

Cells are sensitive to external signals, such as the presence of growth factors, cytokines, pharmacological agents or different types of stress, which result in the activation of signalling pathways that rapidly alter patterns of gene expression. Much research in the signal transduction field has focused on the identification of relevant targets of such pathways. Recently, it has become clear that chromatin structure has a key role in regulating the activation of genes that are connected directly to intracellular signalling. In these processes, the first phase is the induction of immediate-early genes, which are directly connected to intracellular signalling. An intriguing example of how extracellular stimuli are connected to gene activation was shown by F. Posas (Barcelona, Spain), whose group is studying the extracellular signals that lead

to the epigenetic regulation of endogenous genes. The activation of high osmolarity glycerol response 1 (HOG1) in response to salt stress is of special interest because it involves the recruitment of the histone deacetylase reduced potassium dependency 3 (Rpd3) and the deacetylation of the promoter.

Epigenetics and transcriptional chaos in cancer

The cancer cell is a unique context in which transcriptional control is profoundly altered. Two main scenarios of epigenetic alterations are directly involved in this process: marked changes in the DNA methylation pattern of the cell that lead to aberrant histone-modification profiles (Fraga *et al*, 2005); and chromosomal rearrangements that lead to the generation of fusion proteins with aberrant functions, which can also change the histone-modification landscape. This last case is illustrated by the fusion of CREB-binding protein (CBP) to the monocytic leukaemia zinc-finger protein (MOZ) or the MOZ-related factor (MORF), resulting in the CBP-MOZ and CBP-MORF fusion proteins, respectively (Fraga *et al*, 2005).

DNA methylation alterations occur in two directions: hypermethylation of CpG islands that leads to the silencing of tumour-suppressor genes, and hypomethylation of dispersed CpGs in repetitive sequences. It is important to define whether aberrant profiles of CpG island hypermethylation in cancer are a consequence of a targeted process or a random process followed by clonal selection. Elegant studies by P. Jones (Los Angeles, CA, USA), which were obtained by measuring chromatin accessibility to methylation by SssI methyltransferase, provide important clues and new powerful technical tools related to this issue, and emphasize the necessity of bisulphite genomic sequencing to determine DNA methylation patterns.

The existence of specific profiles of CpG-island hypermethylation of tumour-suppressor genes in cancer cells and the search for novel target genes undergoing epigenetic silencing in transformed cells are other important topics in the field. The identification of hypermethylated in cancer 1 (*Hic1*), which codes for a zinc-finger protein, as a potential tumour-suppressor gene with methylation-associated silencing (Chen *et al*, 2003), led the group of S. Baylin (Baltimore, MD, USA) to suggest that this factor interacts with p53 and modulates its activity through the action of the HDACs. P. Pelicci (Milan, Italy) summarized several examples of the connections between epigenetic and genetic alterations in cancer, such as the fusion protein promyelocytic leukaemia–retinoic acid receptor (PML–RAR), which is involved in leukaemia. He also presented important data showing that sensitivity to antitumoral drugs based on the inhibition of histone deacetylases is independent of the transformed cell in connection with the activation of specific death pathways (Insinga *et al*, 2005).

Concluding remarks

Transcriptional regulation is just the tip of the iceberg of epigenetic functions. There are key problems to be solved in the near future, such as the strength of the histone-code hypothesis and the characterization of the complete human epigenome. Many areas are clearly converging as shown by the discussions in this meeting. The cross-fertilization of different fields opens new avenues for our understanding of the maintenance of nuclear and genomic structure and the phenotypic translation of environmental factors.

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