Meeting report

Decoding the epigenetic effects of chromatin

Richard Festenstein and Luis Aragon

Address: MRC Clinical Sciences Centre, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK.

Correspondence: Richard Festenstein. E-mail: r.festenstein@imperial.ac.uk

Published: 24 September 2003

Genome ***Biology*** 2003, **4:**342

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2003/4/10/342

A report on the Alan Wolffe EMBO Workshop on 'Chromatin and Epigenetics', Heidelberg, Germany, 19-22 June 2003.

Alan Wolffe, who died tragically young in 2001, championed the importance of chromatin for gene regulation throughout his career, and this meeting was convened in his memory. As Elizabeth Wolffe - Alan's widow - pointed out, he realized early on that the association of DNA with histone proteins to form nucleosomes, the basic subunit of chromatin, was not merely a way of packaging a large amount of DNA into the nucleus. This meeting brought together friends and colleagues of Alan who are united by their fascination with how cells use chromatin structure to regulate gene expression and who have contributed to our understanding of how this DNA packaging can mark genes epigenetically, thereby functionally and heritably 'labeling' them as active or inactive.

The dynamics of nucleosomes

Karolin Luger (Colorado State University, Fort Collins, USA), who pioneered studies on the structural organization of the nucleosome, presented data on the crystal structure of nucleosome core particles. Using X-ray crystallography and nucleosome-sliding assays to investigate the effects of point mutants of histones H3 and H4, she showed that even subtle disruptions in phosphate-protein interactions correlate with increased sliding rates of the histone octamer with respect to DNA. She also reported that nucleosomes in which H2A was replaced with the variant histone macroH2A (which has only 64% identity to H2A) have an overall structure very similar to nucleosomes containing normal H2A. The differences are concentrated in the L1 loop of the histone, which is thought to be important in ensuring homotypic interactions (which would probably prevent heterodimerization of H2A and macroH2A in the same nucleosome) and in nucleosomal dynamics.

MacroH2A was also discussed by Stefan Dimitrov (Institut Albert Bonniot, La Tronche, France). Previous proteomic studies had shown macroH2A to be localized to the nucleolus. Dimitrov reported that the nucleolar protein nucleolin is required as a cofactor to allow macroH2A-containing nucleosomes to slide and to be remodeled by the SWI/SNF complex. Similarly, Marco Bianchi (University Vita-Salute San Raffaele, Milan, Italy) presented data demonstrating that HMG B1, a high-mobility-group chromosomal protein, facilitates nucleosomal remodeling by the ACF complex. He showed that HMG B1 binds to the DNA just where it enters the nucleosome (in a similar way to the linker histone H1, which associates with the DNA connecting nucleosomes) and may bend the DNA, thus facilitating binding of ACF. Using the technique of fluorescence loss in photobleaching (FLIP), he showed that HMG B1 is highly mobile in living cells. He has also used a combination of fluorescence resonance energy transfer (FRET) and photobleaching to demonstrate that HMG B1 interacts within chromatin with the glucocorticoid receptor, a steroid receptor and transcription factor. In mouse embryonic fibroblasts deficient in HMG B1 there was a reduced response to glucocorticoid.

Moving nucleosomes in order to regulate access to the underlying DNA involves several chromatin-remodeling protein complexes. At present, however, it is unclear how the remodelers induce nucleosome sliding. Two possibilities are the twist-diffusion and looping models; the twist-diffusion model proposes that the DNA screws along the surface of the histone octamer, whereas the looping model suggests that first an internal loop forms and this then translocates around the nucleosome. Jeffrey Hayes (University of Rochester Medical Center, New York USA) has provided evidence against the twist-diffusion model by showing that the human Mi-2 and SWI/SNF chromatin-remodeling complexes can still remodel a positioned nucleosome for which hairpins, flaps and nicks had been introduced into the nucleosomal DNA. Carl Wu (National Cancer Institute, Bethesda, USA)

presented evidence that the *Drosophila* nucleosome sliding complex NURF is required for transcriptional activation or repression *in vivo*. He also showed that mutations of the genes encoding Nurf301 or Iswi (both components of the NURF complex) cause neoplastic transformation of blood cells. Using microarrays, Wu has identified potential candidate genes that are not expressed correctly in *nurf301* mutants and could therefore be responsible for the transformed phenotype. Biochemical and genetic studies of the INO80 complex (a new chromatin-remodeling enzyme) revealed that the actin-related proteins Arp5 and Arp8 participate in chromatin remodeling, possibly by acting as chaperones for histones H3 and H4.

The dynamics of higher-order chromatin

It is becoming increasingly clear that interphase chromatin is highly dynamic and functionally compartmentalized. David Clark (National Institute of Diabetes and Digestive and Kidney Diseases (NIDDKD), Bethesda, USA) reported that induction of the *Saccharomyces cerevisiae HIS3* gene (which encodes a selectable marker) is accompanied by a large scale SWI/SNF-dependent remodeling of chromatin that was indicated by a dramatic loss of nucleosomal supercoiling, a decompaction of the chromatin and a general increase in the accessibility of the chromatin to restriction enzymes. He found that the chromatin remodeling that occurred upon activation of the gene was not restricted to the promoter but involved the whole *HIS3* reporter gene. Susan Gasser (University of Geneva, Switzerland) presented an elegant approach to the study of interphase chromatin dynamics. She has used *in vivo* tagging of yeast chromosomes and video microscopy to show that transcribed chromosomal regions are highly mobile (with quick step movements of up to 0.5 μm) but are constrained to defined subnuclear regions. In contrast, the yeast telomeres and centromeres move in a very restricted area near the nuclear envelope. The mobility of transcribed regions was energy-dependent and was not reduced by inhibition of transcriptional elongation, but was increased either by rapamycin (an inhibitor of elongation) or by the viral transcriptional activator VP16 (which could potentially recruit SWI/SNF complexes). These data suggest that chromatin remodelers such as the SWI/SNF complex are responsible for the high mobility of chromatin.

Wendy Bickmore (MRC Human Genetics Unit, Edinburgh, UK) has used a novel approach to study the higher-order chromatin structure of mammalian chromosomes. She separated 'open' and 'closed' forms of chromatin on sucrose gradients and hybridized the resulting fractions to mammalian metaphase chromosome spreads and microarrays of the whole human genome. Her data reveal a strong correlation between transcriptionally active regions and an open chromatin conformation. She also showed a correlation between nuclear location and activation of a mammalian Hox gene cluster. After induction of embryonic stem (ES) cells to differentiate, this gene cluster was located outside its normal chromosome territory.

It has been proposed that controlled movement of chromatin regions is linked to the regulation of gene expression during development. Along these lines, Frank Grosveld (Erasmus University, Rotterdam, The Netherlands) described direct and dynamic interactions between specific DNase I-hypersensitive sites (HS) of the human β-globin locus control region (LCR) and the promoters within the β-globin locus during development; on the basis of these and other findings he has formulated an 'active chromatin hub' model that takes into account the organization of the β-globin locus in four dimensions (space and time). Moreover, he has shown, for the first time in mammals, that early in development, but not in the adult, the LCR HS 5 can act as a barrier to spreading of what is thought to be heterochromatin. Gary Felsenfeld (NIDDKD), who first described the β-globin insulator element (LCR HS 4) in the chicken, presented data delineating two of its properties: its ability to inhibit enhancer-promoter interactions and to protect against repressive chromosomal position effects. He showed previously that the insulator-binding protein factor CTCF is required for the blocking of enhancer-promoter interactions, and he has now identified β-globin protein 1 (BGP1) as a potential candidate effector of the insulator's barrier function.

Barrier function was also the focus of a talk by Andreas Ladurner (European Molecular Biology Laboratory (EMBL), Heidelberg, Germany). He has investigated the role of the bromodomain protein Bdf1p in *S. cerevisiae* and described how Bdf1p protects acetylated histones H3 and H4 from the Sir2p deacetylase, thereby preventing the spreading of heterochromatin at telomeres and at the mating-type locus. Using chromatin immunoprecipitation (ChIP), he also showed that promoters of genes that normally bind Bdf1p fail to do so in *Bdf1* mutants. Because the binding of Sir3p (a component of transcriptionally repressed heterochromatin in *S. cerevisiae*) to chromatin extends from telomeric heterochromatin into euchromatin in *Bdf1* mutant cells, he suggested that Bdf1p might act as a heterochromatin-euchromatin buffer.

Wolfram Hörz (University of Munich, Germany) presented data revealing a seemingly paradoxical reduction in histone acetylation following gene induction, contrasting with other observations that histone acetylation occurs at transcriptionally active loci. Using yeast strains in which mutations in genes encoding the SWI/SNF complex had caused a delay in activation kinetics, he found that, in fact, transient hyperacetylation occurred, followed by a rapid loss of histones, thereby allowing upregulation of gene expression. A similar finding was presented by Saadi Khochbin (Institut Albert Bonniot), who reported that during the formation of sperm, an increase in histone modifications (histone acetylation and methylation) also preceded histone loss. He also identified a potential function for a gene encoding a protein, BRDT, containing two bromodomains. BRDT is specifically expressed in late spermiogenesis and can condense hyperacetylated chromatin *in vitro*, thus providing a substrate for histone degradation followed by replacement.

Little is known about the DNA sequence requirements for recruiting heterochromatin-mediated silencing in mammals. One of us (R.F.), having previously shown that pericentromeric silencing in mice could be overcome by the human CD2 LCR, showed at the meeting that the short DNA triplet-repeat expansions found in several human diseases can recruit heterochromatin protein 1 (HP1)-sensitive position-effect variegation at multiple sites in the mouse genome. It was also reported that HP1 is highly mobile in the constitutive heterochromatin of living T cells, as detected using fluorescence recovery after photobleaching (FRAP), and that this mobility is increased by immune activation, indicating potential plasticity in the maintenance of heterochromatin domains. Furthermore, late replication of transgene DNA in S phase in T cells correlated with heterochromatin-mediated silencing.

The link between DNA replication and gene expression was also highlighted in the talk by Marcel Mechali (Institute of Human Genetics, CNRS, Montpellier, France), who described experiments suggesting that although the specification of mammalian replication origins needs components of the transcriptional machinery, transcription itself is not necessary. He proposed that such 'epigenetic specifications' might contribute to the regulated formation of chromatin domains (for example in the Hox gene clusters). Continuing the DNA-replication theme, Ron Laskey (Hutchinson/MRC Research Centre, Cambridge, UK) presented striking data indicating that the unwinding of chromatin at the replication fork could be mediated by proteins that rotate the double helix at a more distant location than the replication fork itself. He also showed, using fluorescence microscopy, that the MCM proteins (components of the replication machinery) could be used as an accurate diagnostic tool for cancer.

RNA mechanisms in epigenetic silencing

The emerging evidence for a role of RNA interference (RNAi) in chromatin-mediated silencing has aroused much excitement. Renato Paro (University of Heidelberg, Germany) suggested a link between intergenic transcription in *Drosophila* and Polycomb-mediated silencing (Polycomb is a protein complex that binds to Hox genes, maintaining them in an inactive state), and suggested that this might be due to an underlying RNAi mechanism. He has developed a bioinformatic approach that has identified 167 candidate sequences capable of recruiting either Polycomb (Polycomb response elements or PREs) or Trithorax (the functional antagonist of Polycomb which binds at Trithorax response elements, TREs), several of which were in clusters.

Robin Allshire (The Wellcome Trust Centre for Cell Biology, Edinburgh, UK) presented experiments that establish a link between heterochromatin-mediated gene silencing and RNAi in *Schizosaccharomyces pombe*. He was able to repress reporter-gene expression *in trans* using a complementary hairpin RNA transgene that was homologous to the reporter. Using genetics, he found that silencing was dependent on Clr4 (a homolog of the mammalian and *Drosophila* Suvar39 histone methyltransferase) and involved recruitment of Swi6 (a homolog of HP1, a key component of heterochromatin). To determine whether this mechanism operates normally in wild-type yeast, he looked for and found a correlation between silencing and the proximity of genes to naturally occurring inverted long terminal repeats (LTRs); such repeats generate hairpin RNAs that can cause RNAi.

Asifa Akthar (EMBL) had previously shown that the binding of the chromodomain-containing protein Mof1, which is involved in dosage compensation of gene expression from the male X chromosome in *Drosophila*, is sensitive to RNase treatment. She presented new data showing that not only Mof1, but also Msl3 (but not Msl1), was apparently dependent on RNA for X-chromosome localization in *Drosophila* males. Like Mof1, Msl3 and Msl1 are components of the male-specific lethal (MSL) complex associated with the male X chromosome. In addition, she showed that acetylation of Msl3 at a specific lysine was mediated by Mof1 *in vitro*, and that increased global acetylation induced *in vivo* by the potent histone deacetylase inhibitor trichostatin A decreased the localization of Msl3 to the X chromosome. This acetylation prevented binding of Msl3 to an RNA component of the dosage-compensation machinery, Rox2. Continuing the dosage-compensation theme, Edith Heard (Curie Institute, CNRS, Paris, France) presented interesting data on the temporal regulation of chromatin modifications during female X inactivation in mammals. Using mouse embryos, she showed that X inactivation is initiated much earlier in development than previously thought and well before the first overt signs of differentiation (the appearance of trophectoderm cells at the blastocyst stage). She has shown that coating of the paternal X chromosome with *Xist* RNA at the four-cell stage is followed by exclusion of RNA polymerase II and a simultaneous decrease in acetylation of histone H3 on lysine 9 and in di-methylation of histone H3 on lysine 4, all occurring at the eight-cell stage. From the 16-cell stage onwards, association of Eed/Enx1 (a Polycomb complex containing a histone methyltransferase) begins with both di-methylation of histone H3 on lysine 9 and tri-methylation of histone H3 on lysine 27. This sequence of events provides essential information for understanding X inactivation.

Methylation of DNA and histone H3

Adrian Bird (Institute of Cell and Molecular Biology, Edinburgh, UK) reviewed the *in vivo* functions of the DNA methyl-binding gene (MBD) family in mice, as revealed by gene knockouts. Whereas the knockout of the MBD protein MECP2 in mice mirrored the human Rett syndrome, the knockout of MBD2 (a component of MECP1, the methyl-DNA-binding chromatin-remodeling complex) resulted in maternal disinterest, an inability to repress a methylated transgene reporter, derepression of the interleukin-4 gene in

helper T cells, partial derepression of *Xist* and a dose-dependent effect on intestinal tumor burden (equivalent to the effect of knocking out the DNA methyltransferase Dnmt1). Wolf Reik (The Babraham Institute, Cambridge, UK) described the use of fluorescence microscopy to show that the erasure of DNA methylation that takes place immediately after fertilization in mouse embryos is highly variable and often grossly impaired in 'cloned' embryos, providing a potential explanation for the low efficiency of cloning.

Thomas Jenuwein (Research Institute of Molecular Pathology, Vienna, Austria) clarified the function of the mouse SUV39H and G9a histone methyltransferases using antibodies directed against histone H3 mono-, di- or tri-methylated at lysine 9. He showed that SUV39H could di- and tri-methylate H3 lysine 9 *in vitro* and that pericentromeric heterochromatin was tri-methylated. In contrast, G9a appeared incapable of tri-methylation on this residue. Data presented by Jürg Mueller (EMBL) suggested that the histone methyltransferases Trx and Ash1, which methylate lysine 4 of histone H3 in *Drosophila*, are needed to counteract Polycomb-mediated silencing but are not needed for transcriptional activation *per se*.

It was clear from this meeting that we have begun to decipher the epigenetic mechanisms that help cells 'remember' which genes should be activated or silenced. Hope was expressed by the organizers that this meeting would be the first of a regular series, perhaps alternating with the bi-annual Gordon Conference in Epigenetics, which takes place in New Hampshire (USA).