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Chromatin and epigenetics in development: blending cellular memory with cell fate plasticity

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The epigenetic regulation of chromatin structure and composition has often been studied molecularly in the context of specific DNA-dependent processes. However, epigenetics also play important global roles in shaping and maintaining cell identity, and in patterning the body plan during normal development. Moreover, alterations in epigenetic regulation are involved in many diseases, including cancer. The advances in our understanding of the impact of epigenetics in development and disease were discussed at a recent Keystone symposium.

Introduction

Epigenetics was introduced by Conrad Hal Waddington over 60 years ago as the study of those processes involved in the unfolding of development (Waddington, 1942). The discovery of the role of DNA in inheritance, and of the structure of the DNA double helix, have cast a shadow over this discipline for decades, until it came under the spotlight again in the 1980s with studies on chromatin structure. Epigenetics was then redefined as the study of heritable traits that are not dependent on the primary sequence of DNA (Holliday, 1994). The discovery of the importance of molecular machines that act on chromatin to regulate gene expression has fuelled a great interest in this field. It has recently become clear that epigenetics does not only affect the expression of individual genes. Rather, epigenetic regulators play crucial roles in the global shaping and maintenance of developmental patterning. This involves both dynamic tissue- and cell type-specific changes during patterning, as well as the maintenance of the cellular memory that is required for developmental stability. Furthermore, when epigenetic regulation goes wrong, it can have pathological consequences, which include immune disorders and cancer (Feinberg et al., 2006). Researchers are, thus, increasingly interested in approaching epigenetics from a developmental point of view, as was well illustrated at the 'Epigenetics and Chromatin Remodelling during Development' symposium that was recently organized by Renato Paro and Peter Fraser at Keystone (CO, USA). This meeting highlighted the broad impact that epigenetics has on the regulation of biological processes, much in line with Waddington's original concept, and provided a platform for the communication of major breakthroughs in this field.

Molecular advances in understanding chromatin structure and function

Chromatin is the folded state of genetic material in the cell nucleus. One-hundred and forty-six basepairs of DNA wrap in 1.6 superhelical left-handed turns around an octamer that contains two

copies each of the histones H2A, H2B, H3 and H4, to form the nucleosome. The folding of DNA into nucleosomes affects the function of DNA-binding proteins that have to access their target DNA surfaces. In order to regulate specific DNA-dependent processes, chromatin can be remodelled in three different ways: by removing or mobilizing the histones by means of ATP-dependent nucleosome remodelling machines (Smith and Peterson, 2005); by altering chromatin structure via the post-translational modification of histones (Strahl and Allis, 2000); or by the replacement of specific histones (Henikoff and Ahmad, 2005).

In recent years, the regulatory role of histone modifications, including acetylation, methylation, ubiquitylation and phosphorylation, has emerged as a main player in epigenetic regulatory mechanisms. Specific enzymatic machines are able to mark individual residues of each histone, while other protein complexes are able to read these marks and elicit specific responses (Wang et al., 2004). One example of the language spoken by 'writers' and 'readers' of histone marks in the maintenance of chromatin states was given by David Allis (Rockefeller University, New York, NY, USA). The proteins of the Polycomb group (PcG) and of the trithorax group (trxG) are able to maintain silent and active, respectively, chromatin states at many developmental genes (Ringrose and Paro, 2004). The action of these proteins involves the deposition and the interpretation of multiple histone marks, such as the methylation of specific lysine (K) residues on histone H3 (Ringrose and Paro, 2004). Histone lysines can be mono-, di- or trimethylated. One of the main marks needed to trigger transcription is the methylation of lysine 4 on histone H3 (H3K4). The trxG proteins, called Trx in *Drosophila* and MLL in vertebrates, have been shown to trimethylate H3K4 (Milne et al., 2002; Muller et al., 2002). Recently, the WDR5 protein was shown to act as a reader of the H3K4 di- and trimethyl mark (Dou et al., 2005; Wysocka et al., 2005). The Allis group has now identified a specific reader of the trimethyl H3K4 mark in the largest subunit of the ATP-dependent chromatin remodelling complex called NURF [BPTF/FALZ in humans and the NURF-301/E(bx) complex in *Drosophila*]. Their functional studies indicate that these proteins do not read the dimethyl mark, but only the trimethyl mark, providing an excellent candidate for the factors that act downstream of Trx/MLL to propagate active chromatin states (C. D. Allis, personal communication). The co-crystal structure of a region of NURF-301 bound to an H3 peptide that carries a trimethyl H3K4 has been determined by the laboratory of Dinshaw Patel (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) in collaboration with David Allis. Interestingly, this structure provides an immediate explanation of why NURF-301 specifically reads H3K4 trimethylation but not the trimethylated states of H3K9 or H3K27, which are read, respectively, by the HP1 and Polycomb (PC) chromodomain proteins (Fischle et al., 2003). This specific reading depends on the simultaneous binding by NURF-301 to the lysine in position 4 and to the arginine in position 2 of the N-terminal tail of histone H3, with the additional requirement of a threonine residue in between these two amino acids (no threonine is present between H3K9 and H3K27). These data provide a model to explain the epigenetic maintenance of active chromatin, during which MLL and WDR5 collaborate to produce the efficient trimethylation of H3K4. This trimethylation mark is then recognized by the NURF complex, which remodels the nucleosomes to open chromatin structure at trxG target genes (C. D. Allis and D. J. Patel, personal communication).

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Another way of interpreting active histone marks to drive transcription was highlighted by Steven Henikoff (Fred Hutchinson Cancer Research Center, Seattle, WA, USA). Histones come in different ‘flavours’ – predominant isoforms and histone variants. Histone H3.3 differs from H3 by only four amino acids, but its biology is very different from that of H3. H3.3 marks active chromatin, and it can be deposited on chromatin in a replication-independent manner (Ahmad and Henikoff, 2002). Recently, genome-wide mapping of H3.3 indicated that histone H3 replacement occurs predominantly at sites abundant in RNA polymerase II and methylated H3K4. In addition to H3 replacement, the promoters of active genes undergo a more radical chromatin transition in which histones are stripped from the DNA template (Mito et al., 2005). Moreover, histone H3.3 was found to be enriched on genes located in the hyperactive *Drosophila* male X-chromosome compared with autosomal genes, suggesting that this histone variant might enhance the processivity of RNA polymerase (Mito et al., 2005). Thus, histones can either be modified or removed, perhaps not exclusively, in order to activate transcription.

Chromatin transitions can be dynamically regulated during development. One provocative example of such dynamics was given by Jeannie Lee (Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA, USA), who studies mechanisms of mammalian X-chromosome inactivation (XCI). In XCI, one of the two female X chromosomes is silenced, which equalizes the dose of X-linked gene products in X/X females and X/Y males (Heard, 2005; Huynh and Lee, 2005; Reik and Lewis, 2005). X-chromosome silencing is achieved by upregulating the expression of the *Xist* noncoding transcript, which then spreads in cis on the future inactive X chromosome and induces multiple repressive chromatin modifications that turn off most of the X-linked genes. The Lee laboratory has shown that *Xist* is activated transcriptionally at the onset of XCI. Its transcriptional regulation is mediated by *Tsix*, the antisense partner of *Xist*. On the future active X chromosome,

persistent *Tsix* transcription, a ‘euchromatic character’, paradoxically represses *Xist* transcription. *Tsix* transcription recruits or activates the DNA methyltransferase Dnmt3a, which, in turn, methylates CpG dinucleotides on the linked *Xist* allele and stably represses that allele.

On the future inactive X chromosome, loss of *Tsix* leads surprisingly to a transient heterochromatic state in *Xist* just prior to and during transcriptional induction, with binding of at least one PcG member, accompanied by the trimethylation of H3K27 in the gene promoter and by the decrease in the activating dimethyl H3K4 mark on the *Xist* gene (Sun et al., 2006). This ‘heterochromatic character’ is a paradoxical, but not an unprecedented, condition for an active locus, as some genes can stay active in a heterochromatin environment (Yasuhara et al., 2005). However, in the case of *Xist* this state is reversed in the maintenance phase of XCI; that is, H3K27 becomes hypomethylated, H3K4 becomes hyperdimethylated and H4 becomes hyperacetylated (Sun et al., 2006). Thus, we now know the series of chromatin modifications that pre-empt and determines XCI (see Fig. 1). A future challenge will be to understand how a transient heterochromatin state can result in *Xist* activation, and how the mark is then reversed to the ‘normal’ situation during later stages.

Higher-order chromatin organization and nuclear architecture

Within the cell nucleus, chromatin is not folded in a string of nucleosomes. Depending on the degree of condensation of specific loci, nucleosome fibres can fold further to form the so-called 30 nm chromatin fibre and higher-order chromatin structures, the architecture of which is essentially unknown. These higher-order structures offer possibilities for regulating chromatin domains, as was illustrated by Peter Fraser (The Babraham Institute, Cambridge, UK). The Fraser laboratory has shown previously that multiple genes distributed along the arm of chromosome 7, which contains the β -

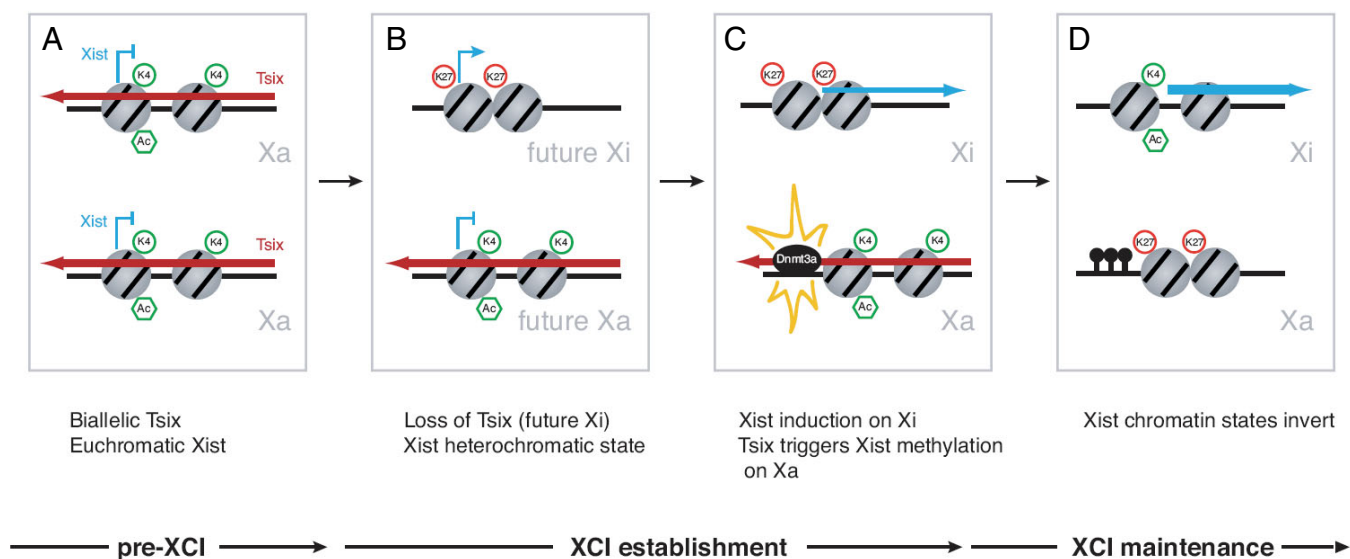


Fig. 1. Model depicting chromatin events during X-chromosome inactivation (XCI). (A) Before XCI, *Tsix* (red) is biallelically expressed and maintains the *Tsix/Xist* locus in an open chromatin state (bearing the active H4 acetyl and H3H4 dimethyl histone marks), which paradoxically precludes *Xist* transcription (blue). (B) At the onset of XCI, silencing of *Tsix* induces local heterochromatinization of the *Tsix/Xist* locus in cis (loss of H4 acetyl and H3H4 dimethyl, gain of H3H27 trimethyl), concomitant with activation of *Xist* in the future inactive X (Xi). (C) On the future active X (Xa), *Tsix* RNA induces Dnmt3a-dependent methylation of *Xist*, locking it into a silent state. (D) Finally, the *Xist* locus switches to a canonical chromatin state, with active marks on the transcribing allele (bottom) and inactive marks on the silent one (top). Reproduced, with permission, from Sun et al. (Sun et al., 2006).

globin locus, can cluster in a so-called transcription factory with the active β -globin domain, when transcribed in erythroid cell progenitors (Osborne et al., 2004). They have now extended this analysis to the study of many loci from different chromosomes. Interestingly, several genes were found to colocalize in transcription factories at high frequencies, suggesting preferential arrangements of specific subsets of genes or networks. In addition to colocalization detected in FISH assays, long-distance interactions were also detected using the chromosome conformation capture (3C) assay (Dekker et al., 2002), suggesting that these interactions might involve molecular contacts among the interacting partners. Further analysis showed that the interchromosomal colocalizations depend, in part, on the preferred neighbour arrangement that some chromosomes have in this specific cell type. However, colocalization is also dynamically regulated as a function of the transcriptional state of the genes and, importantly, transitions from a non colocalized inactive state to a colocalized active state can be rapid (P. Fraser, personal communication). This suggests that, rather than being a passive component in the nucleus, the transcription machinery can represent a potent engine that is able to shape cell nuclear architecture.

On the other side of the coin, silencing machines can also generate long-distance interchromosomal association. This phenomenon was discussed by Giacomo Cavalli (Institute of Human Genetics, CNRS, Montpellier, France), who is studying PcG-mediated silencing in *Drosophila*. PcG proteins silence their target genes via binding to regulatory regions called PcG response elements or PREs. In addition to regulating the closest gene promoter in cis, these elements can also act in trans, as PcG-mediated silencing is often enhanced in the presence of multiple PRE copies (Kassis, 2002). PRE-containing elements, such as the *Fab7* or the *Mcp* regulatory regions from the *Drosophila* Hox locus called the Bithorax complex, can pair in the nucleus even when located on different chromosomes (Bantignies et al., 2003; Vazquez et al., 2006). Components of the RNA interference (RNAi) gene silencing machinery have now been shown to colocalize with nuclear compartments with high concentration of PcG proteins (called PcG bodies) (Fig. 2) and to contribute to the maintenance of long-range nuclear interactions among PcG target elements (Grimaud et al., 2006). As RNAi

components have previously been shown to mediate telomere clustering in *S. pombe* (Hall et al., 2003), one way in which the RNAi machinery might induce chromatin silencing could involve an evolutionarily conserved function in the regulation of nuclear architecture.

Epigenetics in development and disease

The correct deployment of developmental programmes requires both the capacity to progress dynamically through a series of intermediate states, involving the reprogramming of cell fates and the plasticity of cell-cell interactions, and the maintenance of homeostasis (i.e. of a stable response to variable environmental conditions). Several presentations discussed how epigenetic components might contribute towards reaching the best compromise between plasticity and stability during development. PcG proteins are best known for their ability to stably maintain the regulatory states of Hox genes during development. However, the function of these factors can be transiently downregulated by the JNK pathway during tissue injury, as shown by work presented by Renato Paro (Centre for Molecular Biology, University of Heidelberg, Germany). This dynamic response might be crucial to the reprogramming of cell fates, in order to allow cells close to the site of injury to proliferate and to acquire new fates, thus regenerating the complex patterns of the tissue prior to injury (Lee et al., 2005).

The dynamic regulation of PcG proteins occurs not only in flies, but also in plants and in vertebrates. Ueli Grossniklaus (University of Zürich, Zürich, Switzerland) described the complex and dynamic regulation of the *MEDEA* (*MEA*) locus in *Arabidopsis*. *MEA* encodes a homologue of the PcG protein Enhancer of Zeste [E(Z)], the PcG H3K27-specific histone methyltransferase. He showed that *MEA* is able to autoregulate its own promoter in a dynamic parent-of-origin-specific manner: the initial downregulation of the maternal allele around fertilization depends on *MEA* but not on other components of the PRC2-like complex, while the later repressive effect on the paternal allele requires all members of the complex (Baroux et al., 2006).

In vertebrates, PcG factors are not only involved in the maintenance of differentiated cells but also of cellular totipotency (Lessard and Sauvageau, 2003; Gil et al., 2004). Rudolf Jaenisch (Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge, MA, USA) discussed the implications of the genome-wide mapping of the chromosomal distribution of PcG proteins in human and mouse embryonic stem (ES) cells. This was achieved through a combination of chromatin immunoprecipitation with hybridization on DNA microarrays (ChIP on chip) in collaboration with the laboratory of Richard Young (Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge, MA, USA). Their data indicate that PcG proteins maintain ES cell identity by simultaneously repressing a number of developmental transcription factor genes (Fig. 3). Repression correlates with the trimethylation of histone H3K27 and the exclusion of RNA polymerase II from most target genes. Importantly, ES cell differentiation activates the expression of these genes, which occurs concomitantly with the loss of PcG proteins from their regulatory regions (Boyer et al., 2006; Lee et al., 2006). Many of the PcG target genes have previously been shown to be bound by the key transcription factors OCT4, NANOG and SOX2 (Boyer et al., 2005; Loh et al., 2006). This raises the possibility that PcG proteins act as repressors of gene transcription by collaborating with these pluripotency transcription factors to maintain stem cell identity, as well as allowing the reprogramming of stem cell fate by the appropriate differentiation stimuli.

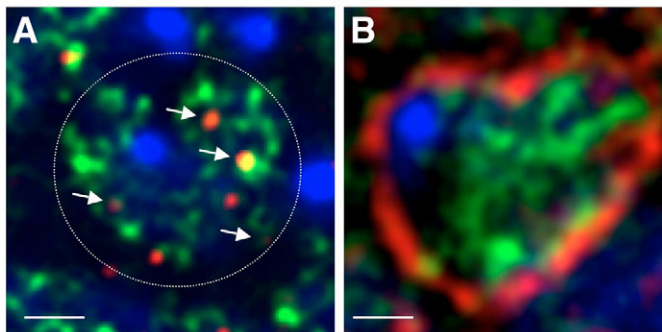


Fig. 2. The relationship between Polycomb group proteins and RNAi components. (A, B) *Drosophila* larval tissues stained with DAPI (blue), Polycomb (green), and Dcr2 (red, A) or Dcr1 (red, B). The Dcr2 RNAi enzyme stains nuclei (the nuclear rim is indicated by a circle) as prominent foci. A substantial part of these foci (arrows) colocalize with Polycomb bodies. This is not always the case for other RNAi components. The right panel shows that Dcr1, which stains the nuclear rim, does not significantly colocalize with Polycomb in the same tissues. Scale bars: 1 μ m. Images courtesy of Charlotte Grimaud and Giacomo Cavalli (Institute of Human Genetics, CNRS, Montpellier, France).

It remains to be seen whether PcG proteins repress their target genes via dedicated DNA elements analogous to *Drosophila* PREs. This is a long-standing issue and no mammalian PREs have been identified to date, but Keji Zhao (NHLBI, NIH, Bethesda, MD, USA) presented his group's studies of human chromosomal regions that are characterized by high levels of histone H3K27 trimethylation. Several of these sites are bound by PcG proteins and

drive gene silencing when tested in functional assays (K. Zhao, personal communication), suggesting that they might represent interesting candidates for human PREs.

Concerning the mapping of PcG protein distribution, Maarten van Lohuizen (The Netherlands Cancer Institute, Amsterdam, The Netherlands) presented a genome-wide analysis of the distribution of *Drosophila* PcG proteins in cultured cells, carried out in collaboration with the laboratory of Bas van Steensel (The Netherlands Cancer Institute, Amsterdam, The Netherlands). They used the DamID technique, in which PcG proteins were fused to Dam-methylase and their sites of binding were mapped by the selective recovery and analysis of DNA methylated by these fusion proteins (Tolhuis et al., 2006). Remarkably, the distribution of PcG proteins in large domains, sometimes many tens of kilobases in size, their tendency to regulate genes that encode transcription factors, and some of the regulatory pathways targeted by these proteins are conserved between mammals and flies. Moreover, an independent ChIP on chip study of the developmental profile of PcG protein distribution in *Drosophila* shows that, similar to vertebrates, PcG proteins associate dynamically with several of their target genes during development (Nègre et al., 2006). This evolutionary conservation suggests that PcG proteins play a pivotal role in driving and maintaining cell fates, maintaining cellular memory and, when necessary, allowing for plasticity of cell fates.

Epigenetic regulation can thus shape developmental landscapes, providing developmental robustness at the same time as allowing adjustment to variable environmental conditions. However, damage to or perturbation of epigenetic components can also result in disease, such as various genetic syndromes or cancer. Stephen Baylin (Johns Hopkins University, Baltimore, MD, USA) discussed cancers that are caused by the hypermethylation, but not the mutation, of the *HIC1* gene DNA. It is known that *HIC1* normally exerts its tumour suppressor function in cooperation with *P53* (*TP53* – Human Gene Nomenclature Database). *HIC1* encodes a transcriptional repressor that requires two protein domains. The Baylin laboratory showed that one of these, the POZ domain, is able to interact with the NAD-dependent SIRT1 histone deacetylase and, together, they repress the SIRT1 promoter. The same complex also deacetylates *P53*, blocking its proapoptotic activity in response to DNA damage (Chen et al., 2005). During the course of aging, *HIC1* is normally hypermethylated. This event might promote *P53* inactivation via upregulation of SIRT1, and this might, in turn, increase the survival of aging cells and their consequent exposure to DNA damage. However, an excessive drift towards the chronic repression of *HIC1* might also increase the risk of neoplastic transformation. This highlights the fact that these processes can be beneficial for health, on one hand, yet they represent intrinsic risk factors for some diseases, on the other (Feinberg et al., 2006).

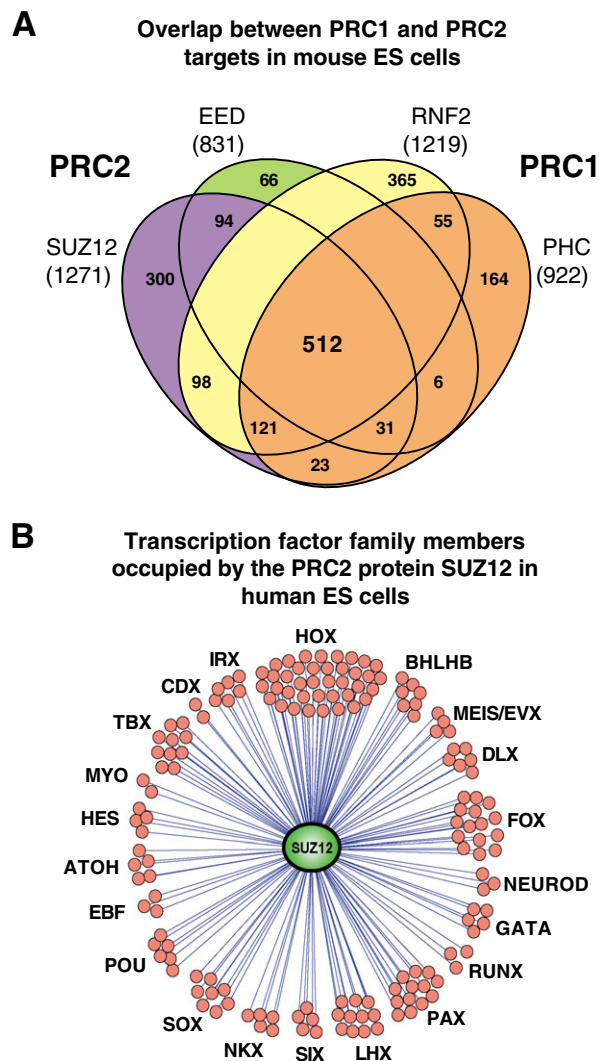


Fig. 3. Mapping the genome-wide distribution of Polycomb group (PcG) proteins in mouse and human embryonic stem (ES) cells. PcG proteins form well-known biochemical complexes: one is PRC2, which contains the H3K27-specific histone methyltransferase EZH2, and its two partners EED and SUZ12; the second complex, named PRC1, contains chromodomain mouse homologues of *Drosophila* Polycomb, which bind to trimethylated H3K27 and to the two interacting proteins PHC1 and RNF2. **(A)** A Venn diagram represents the number of genes bound by each of these components and their overlap, showing extensive colocalization among all members tested (Boyer et al., 2006). **(B)** A similar mapping for human embryonic stem cells identified a large number of transcription factor-coding genes that can be grouped into major developmental transcription factor families (Lee et al., 2006). This illustrates how PcG proteins regulate a variety of crucial developmental patterning processes. Figure courtesy of Rudolf Jaenisch (Whitehead Institute for Biomedical Research and Massachusetts Institute of Technology, Cambridge, MA, USA).

Inheritance of epigenetic states

Another case of epigenetically derived cancer involves an intriguing phenomenon – genomic imprinting – in which only a single allele (either the paternally or the maternally inherited one, depending on the gene locus) is expressed, while the other allele is repressed, a process that mostly involves DNA methylation (Delaval and Feil, 2004). Laurie Jackson-Grusby (Children's Hospital Boston and Harvard Medical School, Boston, MA, USA) discussed an elegant genetic study, performed in Rudolf Jaenisch's laboratory, that involves the consecutive use of Cre and flp recombinases to generate a transient knockout of the DNA methyltransferase gene, *Dnmt1*, in mouse ES cells. In cells that experience a transient loss of DNMT1 function, global levels of DNA methylation are

preserved, while imprinted genes lose their DNA methylation and their parent-of-origin-specific silencing. Embryonic fibroblasts derived from ES cells that have lost imprinted gene expression in this way showed increased growth rate, spontaneous immortalization and the ability to induce cellular transformation that correlates with reduced levels of P19 and P53. Finally, chimeric animals derived from imprint-free ES cells developed multiple tumours (Holm et al., 2005). This demonstrates that the transient loss of a DNA methyltransferase is sufficient to induce heritable loss of imprinting, which can predispose cells to increased risk of neoplastic transformation.

Emma Whitelaw (Queensland Institute of Medical Research, Brisbane, Australia) presented an extreme case of epigenetic inheritance that can be transmitted to subsequent generations in mice. This involves the *agouti viable yellow* (*Avy*) allele of the agouti coat colour locus. Isogenic *Avy* mice display variable expressivity, with coat shades from full yellow, through variegated yellow/agouti, to full agouti (pseudoagouti). Previous work from the Whitelaw laboratory has shown that the expressivity of the agouti phenotype depends on the phenotype of the dam: agouti dams produce offspring with a higher proportion of agouti phenotypes (Morgan et al., 1999). This phenomenon of transgenerational inheritance of chromatin states is present in fungi and in other animal species (Grewal and Klar, 1996; Cavalli and Paro, 1998), and is relatively widespread in plants (Takeda and Paszkowski, 2006). Recently, the Whitelaw laboratory carried out an ENU mutagenesis screen for modifiers of position effect variegation using a GFP transgene. They isolated several mutants that both suppressed or enhanced variegation. Interestingly, in most cases, these modifiers also affect the *Avy* phenotype, with parent-of-origin- and sex-specific effects (Blewitt et al., 2005). This screen might thus prove to be useful for the isolation of mouse genes that are involved in heterochromatin formation and gene silencing. In addition, it seems also to indicate that parent-of-origin-specific effects, and perhaps also transgenerational epigenetic inheritance, might be more widespread in mammals than was previously thought.

Conclusion

The findings presented at this meeting, including the presentations discussed here, together with others that I could not discuss owing to space constraints, show how much epigenetic regulation is intimately linked to developmental processes. Epigenetic factors can both stabilize development by buffering environmental variation, as well as guide the organism through remodelling events that require plasticity of cell fate regulation. Ongoing research will help to clarify how these apparently opposing functions are coordinated, and it is likely to unravel novel molecular mechanisms involved in this regulation. The future is with epigenetics.

I thank the organizers for this fantastic meeting, which included the presentation of a number of exciting new findings, many possibilities for informal interactions among participants and, on top of it, great snow! I also thank Frédéric Bantignies, Charlotte Grimaud, Rudolf Jaenisch and Jeannie Lee for critically reading the manuscript and generously providing figures. I thank all participants who have allowed their unpublished data to be discussed. I apologize to many colleagues whose excellent work I could not discuss here owing to lack of space.

References

- Ahmad, K. and Henikoff, S. (2002). The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* **9**, 1191-1200.
- Bantignies, F., Grimaud, C., Lavrov, S., Gabut, M. and Cavalli, G. (2003). Inheritance of Polycomb-dependent chromosomal interactions in *Drosophila*. *Genes Dev.* **17**, 2406-2420.
- Baroux, C., Gagliardini, V., Page, D. R. and Grossniklaus, U. (2006). Dynamic regulatory interactions of Polycomb group genes: MEDEA autoregulation is required for imprinted gene expression in Arabidopsis. *Genes Dev.* (in press).
- Blewitt, M. E., Vickaryous, N. K., Hemley, S. J., Ashe, A., Bruxner, T. J., Preis, J. I., Arkell, R. and Whitelaw, E. (2005). An N-ethyl-N-nitrosourea screen for genes involved in variegation in the mouse. *Proc. Natl. Acad. Sci. USA* **102**, 7629-7634.
- Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G. et al. (2005). Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947-956.
- Boyer, L. A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L. A., Lee, T.-I., Levine, S. S., Wernig, M., Tajonar, A., Ray, M. K. et al. (2006). Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* (in press).
- Cavalli, G. and Paro, R. (1998). The *Drosophila* Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. *Cell* **93**, 505-518.
- Chen, W. Y., Wang, D. H., Yen, R. C., Luo, J., Gu, W. and Baylin, S. B. (2005). Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. *Cell* **123**, 437-448.
- Dekker, J., Rippe, K., Dekker, M. and Kleckner, N. (2002). Capturing chromosome conformation. *Science* **295**, 1306-1311.
- Delaval, K. and Feil, R. (2004). Epigenetic regulation of mammalian genomic imprinting. *Curr. Opin. Genet. Dev.* **14**, 188-195.
- Dou, Y., Milne, T. A., Tackett, A. J., Smith, E. R., Fukuda, A., Wysocka, J., Allis, C. D., Chait, B. T., Hess, J. L. and Roeder, R. G. (2005). Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* **121**, 873-885.
- Feinberg, A. P., Ohlsson, R. and Henikoff, S. (2006). The epigenetic progenitor origin of human cancer. *Nat. Rev. Genet.* **7**, 21-33.
- Fischle, W., Wang, Y., Jacobs, S. A., Kim, Y., Allis, C. D. and Khorasanizadeh, S. (2003). Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev.* **17**, 1870-1881.
- Gil, J., Bernard, D., Martinez, D. and Beach, D. (2004). Polycomb CBX7 has a unifying role in cellular lifespan. *Nat. Cell Biol.* **6**, 67-72.
- Grewal, S. I. S. and Klar, A. J. S. (1996). Chromosomal inheritance of epigenetic states in fission yeast during mitosis and meiosis. *Cell* **86**, 95-101.
- Grimaud, C., Bantignies, F., Pal-Bhadra, M., Ghana, P., Bhadra, U. and Cavalli, G. (2006). RNAi components are required for nuclear clustering of polycomb group response elements. *Cell* **124**, 957-971.
- Hall, I. M., Noma, K. and Grewal, S. I. (2003). RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. *Proc. Natl. Acad. Sci. USA* **100**, 193-198.
- Heard, E. (2005). Delving into the diversity of facultative heterochromatin: the epigenetics of the inactive X chromosome. *Curr. Opin. Genet. Dev.* **15**, 482-489.
- Henikoff, S. and Ahmad, K. (2005). Assembly of variant histones into chromatin. *Annu. Rev. Cell Dev. Biol.* **21**, 133-153.
- Holliday, R. (1994). Epigenetics: an overview. *Dev. Genet.* **15**, 453-457.
- Holm, T. M., Jackson-Grusby, L., Brambrink, T., Yamada, Y., Rideout, W. M., 3rd and Jaenisch, R. (2005). Global loss of imprinting leads to widespread tumorigenesis in adult mice. *Cancer Cell* **8**, 275-285.
- Huynh, K. D. and Lee, J. T. (2005). X-chromosome inactivation: a hypothesis linking ontogeny and phylogeny. *Nat. Rev. Genet.* **6**, 410-418.
- Kassis, J. A. (2002). Pairing-sensitive silencing, polycomb group response elements, and transposon homing in *Drosophila*. *Adv. Genet.* **46**, 421-438.
- Lee, N., Maurice, C., Ringrose, L. and Paro, R. (2005). Suppression of Polycomb group proteins by JNK signalling induces transdetermination in *Drosophila* imaginal discs. *Nature* **438**, 234-237.
- Lee, T.-I., Jenner, R. G., Boyer, L. A., Guenther, M. G., Levine, S. S., Kumar, R. M., Chevalier, B., Johnstone, S. E., Cole, M. F., Isono, K. et al. (2006). Control of developmental regulators by polycomb in human embryonic stem cells. *Cell* (in press).
- Lessard, J. and Sauvageau, G. (2003). Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* **423**, 255-260.
- Loh, Y. H., Wu, Q., Chew, J. L., Vega, V. B., Zhang, W., Chen, X., Bourque, G., George, J., Leong, B., Liu, J. et al. (2006). The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat. Genet.* **38**, 431-440.
- Milne, T. A., Briggs, S. D., Brock, H. W., Martin, M. E., Gibbs, D., Allis, C. D. and Hess, J. L. (2002). MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol. Cell* **10**, 1107-1117.
- Mito, Y., Henikoff, J. G. and Henikoff, S. (2005). Genome-scale profiling of histone H3.3 replacement patterns. *Nat. Genet.* **37**, 1090-1097.
- Morgan, H. D., Sutherland, H. G., Martin, D. I. and Whitelaw, E. (1999). Epigenetic inheritance at the agouti locus in the mouse. *Nat. Genet.* **23**, 314-318.
- Muller, J., Hart, C. M., Francis, N. J., Vargas, M. L., Sengupta, A., Wild, B., Miller, E. L., O'Connor, M. B., Kingston, R. E. and Simon, J. A. (2002).

- Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. *Cell* **111**, 197-208.
- Nègre, N., Hennetin, J., Sun, L. V., Lavrov, S., Bellis, M., White, K. P. and Cavalli, G.** (2006). Chromosomal distribution of PcG proteins during Drosophila development. *PLoS Biol.* **4**, e170.
- Osborne, C. S., Chakalova, L., Brown, K. E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J. A., Lopes, S., Reik, W. et al.** (2004). Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat. Genet.* **36**, 1065-1071.
- Reik, W. and Lewis, A.** (2005). Co-evolution of X-chromosome inactivation and imprinting in mammals. *Nat. Rev. Genet.* **6**, 403-410.
- Ringrose, L. and Paro, R.** (2004). Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. *Annu. Rev. Genet.* **38**, 413-443.
- Smith, C. L. and Peterson, C. L.** (2005). ATP-dependent chromatin remodeling. *Curr. Top. Dev. Biol.* **65**, 115-148.
- Strahl, B. D. and Allis, C. D.** (2000). The language of covalent histone modifications. *Nature* **403**, 41-45.
- Sun, B. K., Deaton, A. M. and Lee, J. T.** (2006). A transient heterochromatic state in Xist preempts X inactivation choice without RNA stabilization. *Mol. Cell* **21**, 617-628.
- Takeda, S. and Paszkowski, J.** (2006). DNA methylation and epigenetic inheritance during plant gametogenesis. *Chromosoma* **115**, 27-35.
- Tolhuis, B., de Wit, E., Muijters, I., Teunissen, H., Talhout, W., van Steensel, B. and van Lohuizen, M.** (2006). Genome-wide profiling of PRC1 and PRC2 Polycomb chromatin binding in Drosophila. *Nat. Genet.* (in press).
- Vazquez, J., Muller, M., Pirrotta, V. and Sedat, J.** (2006). The Mcp element mediates stable long-range chromosome-chromosome interactions in Drosophila. *Mol. Biol. Cell.* 10.1091/mbc.E06-01-0049.
- Waddington, C. H.** (1942). The epigenotype. *Endeavour* **1**, 18-20.
- Wang, Y., Fischle, W., Cheung, W., Jacobs, S., Khorasanizadeh, S. and Allis, C. D.** (2004). Beyond the double helix: writing and reading the histone code. *Novartis Found. Symp.* **259**, 163-169.
- Wysocka, J., Swigut, T., Milne, T. A., Dou, Y., Zhang, X., Burlingame, A. L., Roeder, R. G., Brivanlou, A. H. and Allis, C. D.** (2005). WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. *Cell* **121**, 859-872.
- Yasuhara, J. C., DeCrease, C. H. and Wakimoto, B. T.** (2005). Evolution of heterochromatic genes of Drosophila. *Proc. Natl. Acad. Sci. USA* **102**, 10958-10963.