Cell. Mol. Life Sci. 64 (2007) 1043 – 1062
1420-682X/07/091043-20
DOI 10.1007/s00018-007-6420-8

Cellular and Molecular Life Sciences

Review

The function of the epigenome in cell reprogramming

C. Lanzuolo and V. Orlando*

Dulbecco Telethon Institute at Institute of Genetics and Biophysics CNR, Via Pietro Castellino 111, 80131 Naples (Italy), Fax +39081 6132702, e-mail: orlando@igb.cnr.it

Received 22 September 2007; received after revision 13 December 2007; accepted 25 January 2007
Online First 8 March 2007

Abstract. During cell differentiation or metabolic switch, cells undergo profound changes in gene expression. These events are accompanied by complex modifications of chromosomal components and nuclear structures, including covalent modifications of DNA and chromatin up to topological reorganization of chromosomes and genes in the nucleus. To various extents, all these levels of organization appear to contribute to the stability and heritability of transcription programmes and define what is meant as the *epigenomic* level of gene regulation. Indeed, damage or perturbation of epigenome components may lead to deviations from a determined cellular programme, resulting in severe developmental disorders and tumour progression. Most recent data also suggest that tissue regeneration and transdifferentiation are controlled by epigenetic functions. Thus, the epigenome provides the molecular basis for the preservation and also for the plasticity of cell identity.

Keywords. Chromatin, epigenetics, nuclear structure, cell identity, reprogramming.

Introduction

As the development of eukaryotic organisms proceeds, chromatin and nuclear structure domains undergo structural modifications and profound topological reorganization. The complex of these modifications appears to integrate the DNA-based coding potential of the genome as they guarantee the mitotic stability of transcription patterns and preservation of cell identity. Thus, each level of chromosome organization, from nuclear compartimentalization all the way through the topological order of DNA elements in the nucleus, the packaging of DNA around nucleosomes and covalent modifications of histone tails and DNA constitutes the epigenome [1, 2] (Fig. 1).
The various developmental programmes drive cells through a series of intermediate states, involving the programming and reprogramming of cell fates, the plasticity of cell-cell interactions and the maintenance of tissue homeostasis.
Epigenome modifications are established early in development and differentiation, but they can also occur later, either by random change or under the influence of the environment [3, 4]. In normal development, germ cells undergo major epigenetic 'reprogramming', involving the removal and substitution of epigenetic marks and, in the sperm, of bulk histones [4]. Upon fertilization, many gametic marks are replaced with embryonic marks and this is a crucial step for the success of early development [5, 6]. Once established and characteristic of epigenome function, a given pattern of modifications should not be changed, unless a specific signal is received that reprogrammes certain transcription patterns. In the adult, misregulation of epigenetic functions leads to alterations in epigenome organization, and this has a role in impacts on tumour progression and stem cell

*Corresponding author.

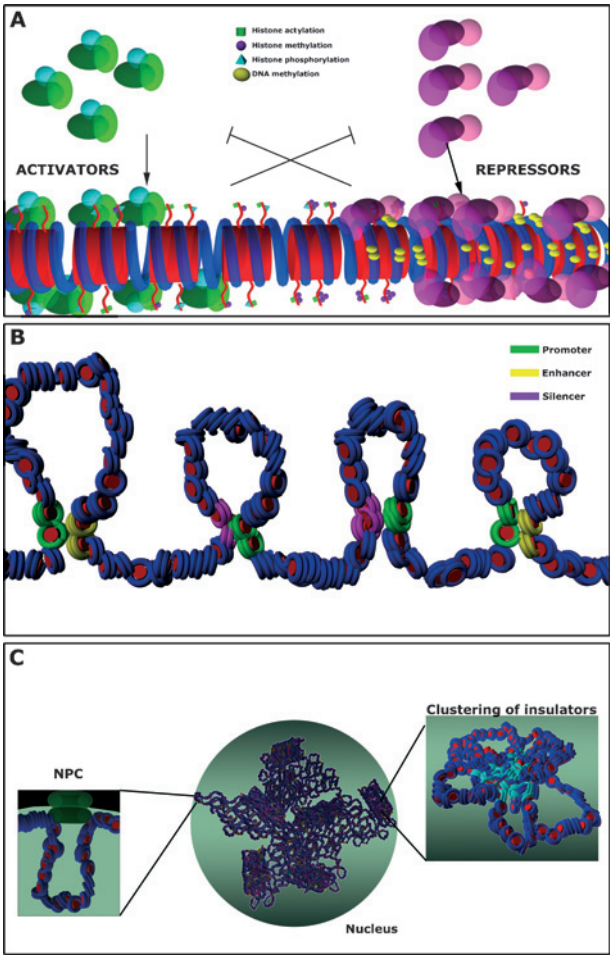

Figure 1. Schematic representation of different levels of epigenome structure organization. (**A**) Posttranslational modifications of histones are differentially interpreted by cellular factors and are involved in the propagation of the transcriptional state through cell division [51, 52]. DNA methylation is typically associated with a repressed chromatin state [19, 20] and seems to play a role in preventing gene reactivation [21]. (**B**) The establishment of chromatin boundaries between promoters and regulatory elements, the formation of topologically distinct chromatin domains, could play a fundamental role in maintaining patterns of silenced or activated genes. (**C**) The positioning and spatial compartmentalization of chromosomes and chromosomal domains plays a central role in gene expression [194–196]. In particular, physical tethering of genomic loci to the nuclear pore complex (NPC) controls their epigenetic activity [158, 215, 216, 219, 220]. In many cases, components of transcriptional silent chromatin are responsible for the positioning to the nuclear periphery [227].

renewal. In addition, mechanisms that control dedifferentiation [7] and change of fate, as in transdifferentiation [8–10], primarily involve reprogramming of the epigenome [11, 12]. Thus, investigation of the mechanisms that control epigenome function are particularly timely in the light of potential applications in tissue repair and regenerative medicine.

DNA methylation

Epigenetic changes include reversible DNA methylation, a postreplication modification predominantly found in cytosine of the dinucleotide sequence CpG [for a review see ref 13]. CpG residues are usually clustered in 'CpG islands' at or near gene promoters [14]. In mammals, analysis of the distribution of DNA methylation reveals that, whereas the majority of cytosines in the context of the CpG dinucleotide are methylated in somatic tissues, CpG islands typically remain unmethylated [15]. In addition, a considerable number of genomic methylated CpGs exist in transposable elements, which are frequently found in intronic regions [16]. Differential methylation of CpGs is tightly linked to flanking promoter activity. Mammals have two types of DNA methyltransferases (DNMTs): the essential, *de novo* methyltransferases DNMT3A/B and the maintenance methyltransferase DNMT1. *De novo* methylation occurs rarely during normal postgastrulation development, but is seen frequently during the setting of imprinting centres at some permanently silenced genes and at genes methylated during ageing and carcinogenesis [17, 18]. In vertebrates, DNA methylation is typically associated with a repressed chromatin state [19, 20] (Fig. 1), and seems to play a role, together with other histone modifications, in preventing gene reactivation [21].

From the mechanistic point of view, DNA methylation can inhibit gene expression by blocking the access of transcriptional activators to their binding site on DNA [22, 23] or by recruiting transcriptional co-repressors [e.g. histone deacetylases (HDACs)]. Added to this, methyl-CpG-binding proteins (MeCPS) mediate transcriptional repression by recruiting heterochromatin-specific proteins to methylated DNA [24–29]. Thus, DNA methylation may trigger a cascade of modifications that contributes to epigenetic silencing. Conversely, in plants, a case has been made for histone methylation preceeding DNA methylation [30]. Thus, the question is open whether DNA methylation is in all cases epistatic to histone modification. Interestingly, with the exception of plants [31], to date no demethylases have been identified. This would in part explain the fact that DNA methylation appears to be the most stable mark in the epigenome.

The role of DNA methylation in higher order structures applies to the chromatin-boundary-element-binding protein (CTCF) in the regulation of the mouse Igf2/H19-imprinted locus. In this context, a differentially methylated CpG acting as boundary element can either block or allow interactions between an enhancer and its promoter when placed between the two elements [32, 33]. CpG methylation

blocks the binding of CTCF to DNA, thus allowing a distal enhancer to stimulate promoter activity across an inert boundary site. Conversely, CTCF can bind the unmethylated boundary element determining a binary switch dependent on DNA methylation of the *Igf2* gene, which is expressed exclusively from the paternal allele [33, 34]. As CTCF is a ubiquitous factor, it is likely that this type of regulation involving topological restriction of enhancer activity would be used at many sites in the genome. However, a possible direct role of DNA methylation in global genome organization remains to be established.

Finally, the epigenetic system based on DNA methylation is widespread among protists, plants, fungi and animals and it does not seem to strictly correlate with genome complexity [35]. An important exception is *Drosophila* in which the existence of this system has been questioned for a long time [36]. Nevertheless, it has been demonstrated that the *Drosophila* methyl-DNA-binding proteins MBD2/3 [37] exist and interact specifically with CpT/A methylation, showing a role in suppressing pericentric position-effect variegation (PEV) [38]. An attractive hypothesis could be that DNA methylation could have evolved as a defence mechanisms acting against the invasion of genomes by transposable elements. Its relics in the mammalian genome, such as Alu, SINE and LINEs, might be primary sites for DNA methylation. As repetitive elements account for almost the 50% of the genome, a major impact of DNA methylation through these sequences on genome organization can be hypothesized.

Histone code(s)

The nucleosome is the elementary unit of chromatin packaging that comprises two H2A-H2B histone dimers and an H3-H4 tetramer wrapped around 146 bp of DNA [for reviews, see refs. 39, 40]. Histone H1 binds 20 additional base pairs and contributes to further DNA/chromatin compaction. The amino and carboxy termini of the histones (histone 'tail'), protruding from the nucleosome, play an essential role in controlling the folding into a higher-order structure, and are the target for posttranscriptional modifications (Fig. 1A), including acetylation [41, 42], methylation [43, 44], phosphorylation [45], ubiquitination [46], sumoylation [47], carbonylation [48], PARylation [49, 50] and others. Adding to the complexity is the fact that the histone tail can carry more than one acetyl or methyl marks at certain amino acid residues. It is now well known that posttranslational modifications of histones play a central role in transcriptional regulation and are potentially involved in the propagation of the transcriptional state through cell division [51, 52]. These covalent modifications are defined as epigenetic 'marks' because they have the potential to produce a specific epigenetic genotype [reviewed in ref. 53].

After Bryan Turner's seminal observation which first revealed that *Drosophila* hyperactive and active X chromosomes, and later mammalian active amd inactive X chromosomes showed a different histone modification profile [54, 55], Jenuwein and Allis [1] proposed that the variety of histone modifications could represent a 'code' that would considerably extend the informational potential of the genetic DNA code [see also refs. 2, 56]. Two models were proposed to explain how histone modifications might alter transcriptional states. One model envisioned a structural role for the modifications such that the resultant charge density of the histone tails would impact on their interactions with the DNA. For example, acetylated histone tails would be expected to propagate a more open chromatin state. The second model proposed that histone modifications might affect transcription by serving as recognition sites for the recruitment of effector modules. Thus the differently modified histone tails would induce different interaction affinities for chromatin-associated proteins, determining gene activation or repression, or cell proliferation or differentiation. The histone code hypothesis has been actively debated in the literature [57]. Genome-wide changes in gene expression were measured on yeast strains in which one, two or three lysines of histone H4 had been substituted with arginine, mimicking the positively charged, nonacetylated lysine state [58]. This study revealed that only the lysine 16 mutation had specific consequences independent of the mutational state of the other lysines [58]. For the lysine 5, 8 and 12 mutations, there were cumulative effects of increased transcription that correlated with an increase in the total number of mutations, suggesting a charge effect on expression more than a lack of a specific histone mark.

Recent studies have shown that site-specific combinations of histone modifications correlate well with particular biological functions. For example, in mammals as well as in *Drosophila*, the tri-methylation of H3K9m3 together with the lack of H3 and H4 acetylation correlates with long-term transcriptional repression [43, 59–64]. In yeast, as well as in human and mouse, H3K4m3 correlates with onset of transcription [65–68], while H3K4m2 resides elsewhere in the vicinity of active genes [65, 69]. In yeast, H2BK123 ubiquitination precedes H3K4 and H3K79 methylation, determining open chromatin and thus active transcription [70, 71]. In the case of histone modifications, however, there exist clear exceptions with

different or even opposing biological consequences. For example, the generally inhibitory H3K9 methylation can in some cases be associated with actively transcribed genes [64, 72, 73], and histone acetylation can be inhibitory rather than stimulatory for transcription [74], depending on the cellular environment. Detailed genomic mapping of methylated H3K4 in human and mouse revealed that in most cases the H3K4-methylated sites are punctate, overlapping precisely active genes, whereas the human HOX clusters contain large H3K4 methylated regions that encompass multiple active genes also covering non-coding regions [65]. The broad profile of H3K4 distribution through the HOX locus could be explained by intergenic transcription specific for this locus that might be required for the maintenance of Hox gene expression, as seen in *Drosophila* [75, 76]. It has been additionally shown that a regulated sequential activation of intergenic transcripts in epigenome structure accompanies the collinear opening of the human HOXA cluster and that this process involved specific changes in epigenome structure of the locus [77]. Thus, intergenic transcription may contribute to epigenome reprogramming.

It appears that instead of a universal histone code there are clear patterns of histone marks that can be differentially interpreted by cellular factors, depending on the gene locus and the cellular context. In a recent report, Bruno Amati and colleagues performed a systematic analysis of histone modifications in the human genome and correlated the profile obtained by chromatin immunoprecipitation with the *in vivo* binding activity of the Myc transcription factor [78]. The striking conclusion of this work is that Myc interaction with its DNA target sites appears to require a specific chromatin structure that is characterized by H3K4/K79 methylation and the pre-engaged basal transcription machinery. Thus the chromatin context can be determinant for transcription factor binding but is clearly not the only determinant.

An important question concerns the role that, DNA sequence might play in epigenetic regulation. By using a computational approach, Widom and coworkers tested a nucleosome-DNA interaction model to predict the genome-wide organization of nucleosomes in yeast [79]. The results show that the yeast genome appears to have an intrinsic nucleosome organization. Indeed, their algorithm, based on DNA sequence, can predict 50% of the nucleosome positions, which is higher than the 35% expected by chance. Interestingly, the most stable nucleosomes would be found immediately downstream of the TATA box and in intergenic regions, but not at functional DNA-binding sites for transcription factors. This is interesting, because histone occupancy could be diagnostic for regulatory regions in the genome. This conclusion would imply a central role for the DNA sequence in regulating nucleosome organization, and thus as a fundamental part of the epigenome. However, these data may not have an adequate level of resolution because they were produced with a bias of 30–35 nt, whereas the exact position of a nucleosome is determined by 3 nt. It remains unclear if the position of nucleosomes is determined by remodelling machines or if these act on a default state situation. Most importantly, the stability and the degree of histone modification (e.g. mono-, di- or tri-methylation) depend on the constitutive presence of specific enzymes and factors that actively counteract opposing forces in the nucleus. What are the factors and the mechanisms that allow the correct action of these enzymes at specific sites in the chromosome? Recent reports claim that various DNA elements in the genome, including epigenetic DNA modules like Polycomb response elements (PREs) appear to be nucleosome-free (see below) [63, 80, 81]. Thus, the possibility exists that certain mechanistic aspects of the recruitment of epigenetic functions at specific sites in the genome would ultimately be directed by DNA sequence.

In conclusion, the concept of a global and absolutely 'deterministic' role for histone modification that would exclude the contribution of DNA sequence in epigenetic regulation remains a matter of debate.

Writers and readers of the histone 'codes'

Chromatin modifiers that are able to 'read' the histone code and to bind covalent modifications of specific nucleosomes represent a fundamental layer of epigenetic gene regulation (Fig. 1A). These factors contain conserved motifs that can recognize a specific histone modification and are thought to regulate the folding of chromatin structure, to maintain an epigenetic state. The conserved proteins heterochromatin protein 1 (HP1) and Polycomb (Pc) provide two well-studied examples. The HP1 protein binds H3K9m2/3 histones through its chromodomain. HP1 self-dimerizes [82] and forms a complex that includes HDACs and the histone methyltransferase Suv39h [83, 84]. This complex can trigger the formation of a higher-order structure and/or a spreading of heterochromatic silencing (PEV). The HP1 protein is primarily associated with silent centromeric heterochromatin and telomeres and is crucial for chromosome condensation and mitotic stability [85–87]. The SUV39H1-HP1 complex is not only involved in heterochromatic silencing but also has a role in repression of euchro-

matic genes by the retinoblastoma protein (Rb) [88] and other co-repressors including origin recognition complex 2 (ORC2) [89]. However, HP1 was also reported to be associated with chromosomal puffs in *Drosophila* where it seems to exert a function on the transcription of heat shock genes after induction [90]. Thus, HP1 may have multiple functions in chromatin, perhaps linked to different histone modification states. Recently, proteins containing a Jumonij C (JmjC) domain were shown to have histone demethylase activity [91]. This activity, by demethylating H3K9m3, might compete with silencing mechanisms present at heterochromatic regions. This finding is crucial because it suggests that modifications in the genome are most likely the result of a regulated equilibrium between opposing forces and not simply the perpetuation of a default state. This would result in a tight control but a dynamic degree of plasticity for the cell.

Stabilization of constitutive heterochromatin compartments is necessary for proper chromosome segregation, inhibiting potentially deleterious recombination between homologous repetitive sequences, and it participates in the developmental regulation of specific genes. Thus initiation and maintenance of heterochromatic structures appears to be an essential aspect of genome physiology. Recent work indicates that heterochromatin formation depends also on RNAi components acting in chromatin, that would trigger histone methyl transferase (HMT) recruitment, H3K9 methylation and Swi6/HP1 binding [92]. In tune with an RNA-dependent mechanism, it has been shown that low levels of transcription are required at 'silent' heterochromatic sites to produce the substrate for the RNAi machinery acting co-transcriptionally with RNA polymerase II (Pol II) [93]. Thus, we are confronted with an apparent paradox, as transcription through a silent locus would be required to keep that locus silenced. Strikingly, the yeast Swi6/HP1 protein was shown to recruit Epe1, a JmjC domain protein that appears to counteract HMT activity, allowing Pol II to move along heterochromatic domains [94]. Thus, Swi6/HP1 may be a fundamental switch molecule that would fine-tune the initiation and maintenance of heterochromatin by determining the rate of movement of Pol II. Thus regulated transcription would control heterochromatin compartments.

The establishment of distinct chromatin domains can be achieved through incorporation of nucleosome-binding, non-core histone proteins, such as the linker histone H1, HP1 and poly(ADP-ribose) polymerase 1 (PARP-1). PARP-1 is the most abundantly expressed member of a family of proteins that catalyse the transfer of ADP-ribose units from NAD^+ to target proteins [95, 96]. Early biochemical studies suggested that PARP-1 might be implicated in the decondensation of chromatin by PARylating histones and destabilizing nucleosomes [50]. Further studies have demonstrated PARP-1-dependent accumulation of polyanionic poly(ADP-ribose) (PAR) at decondensed, transcriptionally active loci in native chromatin [97]. These data support a model whereby PARP-1 promotes chromatin decondensation by PARylation of histone H1. More recent studies indicate that PARP-1 has specific nucleosome-binding properties that can alter transcription by altering chromatin structure, in a manner similar to the linker histone H1 [98]. The incorporation of PARP-1 into chromatin promotes the formation of higher-order chromatin structures that localize to discrete transcriptionally repressed chromatin domains in vivo [98]. In the presence of NAD^+, PARP-1 autoPARylates and dissociates from chromatin, resulting in the formation of decondensed, transcriptionally active chromatin structures [98]. Thus PARP-1 appears to be an important regulator that can facilitate both the compaction and decondensation of chromatin depending on the physiological signal available.

Pc is the founder member of the Polycomb group (PcG) of proteins, which control the repressed state of developmentally regulated genes in an epigenetic manner [99]. The evolutionarily conserved PcG act in large complexes that are able to write and to read the histone code: Polycomb repressive complex 1 (PRC1) and PRC2 (also known as ESC-E[Z]) [100, 101]. The PRC2 complex contains an intrinsic HMTase activity, enhancer of zeste [E(z)], that preferentially trimethylates H3-K27, which in turn recruits Pc [102–104]. In fact, *in vitro* the chromodomain of Pc protein has the highest affinity for H3K27m3 and Pc binding to chromatin is impaired in E(z) mutants, suggesting a direct role for H3K27m3 in Pc function *in vivo* [102, 103].

In *Drosophila*, the PcG proteins exert their function via specialized modular DNA elements called PREs that, in combination with core promoters, are necessary to prevent changes in early determined transcription states of developmentally regulated genes [105, 106]. Characteristic of PREs is that upon the switch to the repressed or activating modes, determined during early embryogenesis, they stably maintain promoter states throughout development and adulthood [105]. Bioinformatic analysis of PcG binding sites led to the identification of a sequence motif able to generate predictive patterns of PRE distribution in the *Drosophila* genome [107]. Interestingly, the same sequence is found at core promoters, suggesting that these may contain 'weaker' PRE consensus sequences [107, 108]. Indeed, PcG com-

plex(es) interact *in vivo* with PREs and core promoters [109–111].

Genome-wide analyses performed in different laboratories have shown that ChIP on CHIP binding profiles of PcG proteins correlate with those obtained for H3K27m3 [112]. These data strongly suggest a role for H3K27m3 in long-range effects mediated by PcG proteins. Moreover, upon activation, changes in epigenome structure take place at core PREs and the active promoters only [63, 113]. However, these studies also show that, at least in *Drosophila*, histone methylation alone could not be sufficient for the recruitment of Pc [113]. Indeed, the sharp binding profile of the E(z) HMT responsible for the H3K27 methylation does not correlate with the broad profile of H3K27m3 distribution. In addition, the strong Pc binding peaks at PREs are often associated with low values in the histone methylation profile, probably due to low levels of nucleosome occupancy [113]. Recent work by Verreijzer and co-workers showed that DNA sequence and a specific subset of DNA-binding factors appear to be the primary determinants for the recruitment of the PRC1 complex to PREs [81]. These results indicate a role for DNA sequence also in Pc-mediated epigenetic silencing.

PcG proteins are found in discrete bodies in the nucleus [114, 115]. The molecular nature and regulation of Pc bodies remain to be elucidated. Broader biochemical analysis of the PRC1 complex revealed interactions of PcG proteins with general transcription factors (GTFs), condensins, topoisomerase II and RNAi components [109, 116, 117; F. Cernilogar, A. Breiling and V. Orlando, unpublished]. The latter appears to influence chromosomal pairing and PcG-mediated long-term memory [118]. Moreover, *in vivo* analysis by 3C and FISH-I show that PREs give rise to a multiloop superstructure that is required to maintain silencing (C. Lanzuolo et al., unpublished). Thus other mechanisms are likely to complete the function of PcG proteins that act at higher levels of chromosomal organization (see below).

Recently, the HMT enhancer of zeste 2 (EZH2) was shown to interact *in vivo* and *in vitro* with DNMTs. This interaction is needed to bring DNMTs to EZH2 target promoters, and depletion of EZH2 impairs this recruitment, leading to derepression of target genes [119]. Although we already knew that functional DNMT1 is required for the formation of intact PcG bodies [120], this is the first direct evidence that links PcG-mediated repression to DNA methylation [119]. Thus the hierarchy between writers and readers can be different from one case to another.

A further important aspect concerns the intracellular regulation of enzymatic activities linked to the epigenome. In particular, EZH2 function appears to be controlled transcriptionally and posttranscriptionally. A number of reports indicate that that hyper-expression of EZH2 causes tumour progression [121]. Two intriguing examples are provided by recent findings about the HMT EZH2. Levels of H3K27m3 and EZH2 enzymatic activity were shown to be dependent on the phosphoinositide 3-kinase-Akt (PI3K-Akt) signalling pathway [122]. In mammals, the EZH2/PRC2 complex was found to localize also in the cytoplasm of the T cell and its function was required for actin polymerization and cell differentiation [123]. However, to date, the putative cytoplasmic target of the EZH2 complex remains unknown. Thus, complex gene networks contribute to the control of HMTs that might initiate in the cytoplasm and end in chromatin. Importantly, the identification of signalling pathways that control chromatin structure may provide the entry key for farmacological control of epigenome reprogramming (see below).

Histone dynamics and chromatin opening

While methylation of H3K27 and H3K9 is often associated with transcriptional silencing, the same modification of K4 of histone H3 is recognized by structurally distinct protein domains that mediate opposite biological functions [124–130]. Recent work reveals that the plant homeodomain (PHD) is a highly specialized methyl-lysine-binding domain [125, 126, 128, 130], able to read the H3K4me3 mark. This PHD domain is present in multiple chromatin-associated proteins [131], often in conjunction with a bromodomain, a module that recognizes acetylated histone tails, first identified in the Brahma protein, the motor protein of the SWI/SNF remodelling complex [132–134]. A precise hierarchy between remodelling and histone modification was recently established, providing a proof-of-concept for readers and writers in chromatin. The role of the PHD domain of the nucleosome-remodelling factor NURF was shown to be essential for faithful activation of developmental genes in guiding the remodeller onto chromatin through the H3K4me3 mark [130]. Alterations in this NURF/H3K4me3 binding compromise spatial control of *Hox* gene expression during *Xenopus* embryonic development [130]. Conversely, the PHD domain of the tumour suppressor protein ING2 (inhibitor of growth) recognizes both H3K4me3 and H3K4me2 and represses proliferation genes [128]. In response to DNA damage, ING2 stabilizes the binding of histone deacetylase complexes that, by removing the nearby acetylation marks, trigger the formation of a closed chromatin structure in which the genes are then repressed [128]. This pathway constitutes a new

mechanism by which H3K4me3 functions in repression.

In addition to covalent histone modifications, there is another mechanism that has been implicated in transcriptional regulation and in the control of epigenome inheritance: histone replacement [for a review see ref. 135]. All eukaryotic genomes encode four conserved core histones that package bulk chromatin, but a fraction of chromatin contains one or more variant isoforms of the canonical histones. Some variants exchange with the pre-existing histones during development and differentiation [136–139], by a mechanism called replication-independent nucleosome assembly [for a review see ref. 140]. This replacement often results in the variants becoming the predominant species in the differentiated cell. These observations have led to the suggestion that the histone variants have specialized functions regulating chromatin dynamics. In *Drosophila*, the H3 and H3.3 histones are extremely similar, differing by only four amino acid residues [141]. The histone H3.3 variant localizes to gene-rich chromatin in *Drosophila* cells [142] and on transcriptionally active genes [143–145]. High resolution mapping of nucleosome variants confirms that there is a clear association between H3.3 and covalent histone modifications associated with active chromatin [146], even at the level of single genes. Moreover, this H3 variant is enriched for the presence of 'marks' that reflect transcriptional competence, such as di- and tri-methylation of K4, acetylation of K9, K18 and K23, and methylation of K79 [147].

In several organisms, H2AZ, a histone H2A variant, is essential for correct development and is involved in the activation of gene expression [148–150]. Studies in yeast indicate that Htz1, the yeast orthologue of H2AZ, localizes within euchromatic regions, flanked by heterochromatin, restricting the spreading of silent chromatin [151]. In particular, Htz1 occupies the promoters of both active and inactive genes in euchromatin [152]. Importantly, gene activation promotes Htz1 loss, while gene repression promotes its deposition [153, 154]. The incorporation of Htz1 at inactive promoters has been proposed to serve as a stable epigenetic mark, keeping promoters in a repressed state until the appropriate activation signal is received [153, 154]. In mammals, H2AZ, found at pericentric and heterochromatic regions [138, 155], is also involved in the maintenance of facultative heterochromatin confining HP1α to specific regions [156]. Thus, replication-independent assembly, in addition to covalent histone modification, could be one of the epigenetic mechanisms essential for the propagation of transcriptionally programmed chromatin [142, 143, 145].

Epigenetic regulation and chromosomal 3D interactions

An emerging aspect of the complexity of the epigenetic information concerns the role that *cis-trans* interactions, occurring between different regulatory elements of the genome, their topological organization and their association with specific components of the eukaryotic nucleus, might play in maintaining transcriptional programs [157–159]. Genes with highly complex expression patterns are controlled by a large set of enhancers, insulators or repressors, which are often located at a considerable distance from the regulated gene. Thus, the establishment of chromatin boundaries between promoters and regulatory elements, the formation of topologically distinct chromatin domains, could play a fundamental role in maintaining patterns of silenced or activated genes (Fig. 1B).

Insulators have been implicated in defining the junctions between structural domains [160]. DNA elements with insulator function are found in most eukaryotes, suggesting that these elements (but not their sequence) have a conserved role in organizing transcriptional domains. Two classes of insulators have been identified that differentially affect transcriptional processes: (i) enhancer blockers, which prevent enhancer-dependent transcription when placed between an enhancer and promoter and (ii) insulator barriers, which impede the spread of heterochromatin emanating from an initiation site [160].

Evidence suggesting a role for insulators in higher-order chromatin structure has been provided by the analysis of the *gypsy* insulator present in about 25 specific locations in the *Drosophila* genome [161]. *Gypsy* insulators, separated at a distance in the genome, may come together and form large insulator bodies in the nucleus during interphase. These aggregates represent higher-order structures of chromatin implicated in the regulation of gene expression [161]. Global changes in transcription, induced by a heat shock, determine a dramatic modification in the distribution of the insulator proteins and DNA [161, 162]. Using FISH analysis of high-salt-extracted nuclei, it has been shown that DNA sequences located between two *gypsy* insulators form a loop in interphase nuclei, while placement of a new insulator in the middle of the loop results in the formation of two smaller loops [162]. These higher-order chromatin structures are dependent on functional insulator proteins and an intact nuclear matrix [162]. Thus, chromatin loop formation may be a mechanism used by insulators to functionally compartmentalize the genome [163].

Recent work by the Grewal laboratory analysed the

function of the yeast inverted repeat (IR) insulator that flanks the *mat* locus and defines a sharp chromatin modification boundary [164]. The IR element contains a B-box, the high-affinity binding site for the RNA pol III transcription initiation factor TFIIIC. The B-box was shown to be essential for boundary function along with TFIIIC and the production of intergenic transcripts from the IR element. Important information was provided by the genome-wide and immunofluorescence analyses of TFIIIC *in vivo*. ChIP on CHIP revealed a distinct pattern of 67 loci dispersed throughout euchromatic regions of the genome, in which high levels of TFIIIC were present but pol III was absent. Immunofluorescence revealed five to 10 TFIIIC bodies that localize to the nuclear periphery in proximity to the nucleolus. Importantly, DNA sequences of the *mat* region, centromeres and the 67 loci were found in these TFIIIC bodies. The latter finding is reminiscent of the localization of other insulator proteins that were proposed to establish chromatin loops to define independent domains of transcriptional activity [160]. In mammals, about 500 000 copies of Alu repeats, which account for 5% of the genome content, contain B-box sequences [165]. This large fraction of potential TFIIIC-binding sites indicates a potential role for at least a fraction of Alu elements in the spatial organization of the mammalian genome.

Bringing *cis* elements together: looping

The looping model also has been adopted to describe the multiple regulatory mechanisms of transcription occurring at complex loci like the homeotic gene clusters. The *Drosophila* bithorax complex (BX-C) contains three homeotic transcription units, *Ultrabithorax* (*Ubx*), *abdominalA* (*abdA*) and *AbdominalB* (*AbdB*), each regulated *in cis* by 60–80 kb of DNA, including promoters, enhancers and insulators. The spatially restricted expression patterns of the homeotic genes, set in the early embryo, are maintained by the PcG and trxG bound at PREs [106]. A key aspect of PRE function is its ability to interact with homeotic gene promoters [108]. It has been shown that an insulator can block interactions between an enhancer and its promoter when placed between the two elements. Conversely, two flanking insulators can induce enhancer-promoter interactions [166]. Similarly, one insulator located between a PRE and the promoter can block the spreading of PRE-bound proteins, but two neighbouring insulators facilitate the association of PRE-bound PcG complexes with the promoter [167]. This suggests a looping mechanism where the pairing between two insulators might bring the target promoter in contact with PRE-associated PcG proteins [166, 167].

Endogenously, insulators, which often overlap PREs, can contribute to the network of *cis* interactions between PREs and BX-C gene promoters. Recently, direct looping between BX-C regulatory regions in both the active and the repressed state has been described. RNA FISH analysis suggests that distal enhancers often loop to the *AbdB* promoter region and frequently associate with the *AbdB* transcription unit located on the homologous chromosome [168]. On the other hand, in transgenic flies, the targeting of the Dam methyltransferases (Dam) to the *Fab-7* insulator results in a strong methylation signal at the *AbdB* promoter, suggesting a long-distance physical interaction between distal regulatory elements [169]. The formation of this loop is dependent on the repressed state of *AbdB*. Thus, physical insulator/insulator or insulator/promoter interactions suggest that epigenetic DNA elements might create higher-order structures relevant for the epigenetic inheritance of homeotic genes.

Due to technical limitations, it is not possible to distinguish between stable or transient interactions between regulatory elements. Other technologies, like Fluorescence resonance energy transfer (FRET) or Fluorescence Recovery After Photobleaching (FRAP) suggest that any interaction in the nucleus is highly dynamic [170]. This confirms the intrinsic but dynamic metastable nature of active or inactive genes. In the cell, PRE function appears to be strictly dependent both on *cis* and *trans* interactions. A typical, physiological feature of PREs is that its silencing function is enhanced by *trans* interactions among chromosomes [171, 172]. Indeed, it was postulated that the maintenance of silencing would require the concerted action of 'strong' and 'weak' PREs present in *cis* in the BX-C [173]. Using *in vivo* microscopy, it has been shown that one of the BX-C PREs, *Mcp*, is able to mediate physical interactions between distant chromosomal regions [174]. Strikingly, *trans*-sensing effects were reported, in which a PRE construct would lose its epigenetic function in subsequent fly generations, when combined in the F_0 with specific deletions of the corresponding endogenous element [171].

In mammals, the transcription factor promyelocytic leukaemia zinc finger (PLZF) might be one of the *trans*-acting factors that mediates the formation of higher-order structures to control the expression of homeotic genes. Indeed, PLZF mediates the transcriptional repression of HoxD genes *in vivo*, directly binding Hox regulatory elements and tethering the PcG protein Bmi1 [175]. Moreover, it mediates the formation of DNA loops *in vitro*, holding together

distant PLZF-binding sites located within the homeotic cluster [175].

Direct evidence for physical association between mammalian chromosomal regulatory regions *in vivo* has been found for the murine β-globin gene cluster [176, 177]. When actively expressed, the *cis*-regulatory elements of the *β-globin* locus are in proximity in the nuclear space, forming a compartment termed the active chromatin hub (ACH) [176, 177]. The transcription factors GATA-1 [178] and EKLF [179] are required for ACH formation, while CTCF regulates the local balance between active and repressive chromatin marks [180]. These studies suggest that transcription factors, together with insulators, can play an essential role in the three-dimensional organization of gene loci.

Regional co-ordination of gene expression is also found in many imprinted gene clusters in the mammalian genome and is regulated by imprinting control regions (ICRs) [181, 182]. One of these clusters contains the paternally expressed *Igf2* and the maternally expressed *H19* gene. Both genes share the same enhancers, and the ICR is located in the 5′ flank of the *H19* gene. Using the 3C technique [183], Reik and colleagues demonstrated that on the paternal chromosome, the DMR2 loops out to interact with the methylated ICR. This event would push the *Igf2* promoter to contact the *H19* enhancer, triggering *Igf2* transcription. On the maternal chromosome, another differentially methylated region, DMR1, interacts with the unmethylated ICR, partitioning the *Igf2* promoter into a silent loop. This configuration inhibits *Igf2* transcription, promoting instead *H19* transcription [184]. This structure, maintained also during mitosis [185], depends on the binding of CTCF to the maternal ICR [186], which prevents *de novo* methylation [187, 188]. In the absence of CTCF, the binding site becomes methylated and can no longer bind CTCF, reverting the higher-order structure of the region from a 'maternal' (repressed) to a 'paternal' (active) conformation of the *Igf2* gene [186]. Recently, it has been shown that CTCF, in addition to its role mediating the formation of chromatin loops, also promulgates *trans* interactions between specific transcriptionally active regions of chromosomes 7 and 11 [189].

Further evidence for a role of chromatin loop structures for the transcriptional status of chromatin domains has been reported for the *Dlx5* and *Dlx6* loci, which are target sites for MeCP2. The higher-order structure at the *Dlx5–Dlx6* locus consists of different looped domains, which reflect the transcriptional status of the genes. Chromatin immunoprecipitation-combined loop assays showed that MeCP2 mediates the formation of the silent 11-kb chromatin loop at the *Dlx5–Dlx6* locus. This loop was absent in chromatin of brains of *Mecp2*-null mice, and *Dlx5-Dlx6* interacted with far distant sequences, forming distinct active chromatin-associated loops. These results show that MeCP2 regulates transcription by promoting the formation of a silent-chromatin loop [190].

The special AT-rich-sequence-binding protein 1 (SATB1) is a transcriptional regulator controlling gene expression that is essential in the maturation of the immune T cell [191]. This factor binds to the nuclear matrix attachment regions of DNA, where it recruits histone deacetylase and represses transcription through local chromatin remodelling [192]. Recently, a role for SATB1 in the formation of active higher-order structure has been suggested [193]. Upon T_H2 activation, SATB1 induces the formation of a unique transcriptionally active chromatin structure at the cytokine locus. In this structure, chromatin is folded into numerous small loops, all anchored to SATB1, indicating that SATB1 functions as a cell-type-specific 'genomic organizer'.

The role of nuclear architecture: large-scale chromosomal interactions

Several studies have described changes in large-scale chromatin organization upon transcriptional activation and repression. In mammals during lymphocyte differentiation, the Ikaros repressive complexes determine repositioning of inactive genes (e.g. globin genes) at pericentromeric heterochromatin [200]. This spatial organization is a feature of the cycling lymphocytes that is acquired by resting non-cycling cells to repress specific genes, as they enter the cell cycle [201]. These seminal observations led to a model in which association with pericentric heterochromatin would be a condition for maintenance of gene silencing [200, 201].

The gene-dense major histocompatibilty complex locus has been reported extend away from chromosome territories, and the incidence of this correlates with transcription from the region [202]. A similar result has been seen for the genes of the epidermal differentiation complex [203]. Targeting of the transcriptional factor VP16 to a specific chromosomal site determines changes in large-scale chromatin including unfolding of heterochromatin and recruitment of transcriptional machinery [204]. On the other hand, *in vivo* HP1 targeting has an opposite effect on large-scale chromatin conformation, determining enhanced tri-methylation of H3K9 and local condensation [205].

Using an integrated cytological system observed in real time, Janicki et al. [144] investigated how gene expression co-ordinated spatially and temporary in

living cells. From the time that a gene is induced until after the rate of transcription has peaked, local decondensation is accompanied by profound changes in chromatin composition. They found a dynamic loss of HP1 and concomitant reduction of levels of H3K9 methylation and a recruitment of the histone variant H3.3. These results suggest that higher-order chromatin structure could play a role in transcriptional regulation, ensuring heritable gene expression.

A further example was provided by studies on the spatial-temporal regulation of the mammalian *HoxB* cluster. In mouse ES cells, a fully repressed *HoxB* cluster would occupy a defined and restricted small area of the nucleus. Upon collinear activation of the *HoxB* cluster, triggered by the retinoic acid morphogen, active *HoxB* genes loop out of the repressed chromosome territory [157]. This event was also observed along the anterior-posterior axis of developing mouse embryos, in neural tube nuclei [206]. This relocalization does not precede *HoxB* expression. As proposed by Bickmore and colleagues, *Hox* genes could migrate, after their transcriptional activation, to foci enriched in RNA pol II. Importantly, in the same study, it was shown that in trichostatin A (TSA)-treated ES cells, hyperacetylation of the mammalian *HoxB* cluster was not sufficient to either activate *HoxB* genes or to relocate modified regions of the cluster away from heterochromatic domains [157]. These findings strongly suggest that relocation of clustered genes occurs when *Hox* genes are actively transcribed. The key question to be answered is what are the factors that determine the change of positions inside the nuclei.

Structural analysis of active versus inactive chromosomal domains reveals a less sharply defined picture than expected. ChIP data on a variety of gene systems and also analysis of chromatin modification depict a scenario in which in many cases activators and repressors co-habit, though at different levels, at the same sites. Analysis of compact and open chromatin fibres across the human genome by hybridization of metaphase chromosomes and genomic microarrays revealed that there is no simple separation of euchromatin and heterochromatin [207]. The same study showed that many transcriptionally inactive genes are in open chromatin fibres and vice versa. The conclusions of these experiments are mirrored by the results obtained by measuring the rate of transcription initiation and the engagement with pol II sites of active and inactive genes [208]. In this case, it was shown that active globin genes are not active all the time and that their relative active state is proportional to the rate of association with pol II 'factories' in the nucleus. Thus an On-Off mechanism appears to regulate the ability of genes to be transcribed. Conversely, certain silencing mechanisms appear to require transcription, and thus active pol II can be present at silenced loci. This is the case of the production of pol-II-dependent, non-coding transcripts (subsequently processed by RNAi) that are required for heterochromatin formation and mitotic stability [93]. Other cases are also known in which production of non-coding RNA that is transcribed from an upstream region through the promoter of a silenced gene is required to maintain silencing [209]. Thus regulated engagement with pol II foci and the correct topological order of regulatory regions might also determine the maintenance of gene silencing.

Finally, nuclear compartments defined by a high concentration of specific factors could also play a role in co-ordinating the expression of genes that are co-regulated, but are separated by longer chromosomal regions or are located on different chromosomes. During differentiation of T cells, specific genetic loci that regulate transcription of cytokine-encoding genes associate physically in the nucleus through intra- and inter-chromosomal interactions, suggesting that many genes could share the same subnuclear compartment in the nucleus thanks to their chromatin repertoire of regulatory factors [210]. Emerging technologies like circular chromosome conformation capture (4C) coupled to microarrays might provide the ideal tool to investigate the topological organization of specific groups of genes in the eukaryotic nucleus [211]. There are many other examples of nuclear compartments/bodies whose function remains to be elucidated. In all cases, nuclear structures appear to be highly organized and dynamic at the same time.

Living in the centre or at the periphery

The positioning and spatial compartmentalization of chromosomes and chromosomal domains play a central role in gene expression [194–196] (Fig. 1C). In interphasic nuclei, each chromosome appears to localize in a limited and specific space that has been defined as 'chromosomal territory' (CT) [197]. Chromosomes with highest gene density are preferentially disposed toward the nuclear interior, and gene-poor chromosomes locate towards the nuclear periphery [198]. However, individual human genes can be transcribed from within the interior of chromosome territories that are not located in the nuclear centre [199], suggesting that the basal transcription machinery can gain access to the chromosome interior.

The nuclear periphery is a complex structure that includes large regions of condensed chromatin associated with the nucleoskeletal lamina and the nuclear pore complexes (NPCs). In *Drosophila*, chromosomal

regions enriched in inactive chromatin are found in close proximity to the nuclear lamina [212, 213]. In the same biologycal system, induction of gene expression or active histone marks reduces the association with the lamina [213].

Localization of genes at the nuclear periphery is not strictly correlated with transcriptional repression [158]. Many genes interact routinely with the NPC basket and this represents an early step in gene activation [214] (Fig. 1C). In particular, boundary elements interact with the NPC and various proteins involved in nuclear-cytoplasmic traffic, such as the exportins Cse1p, Mex67p, and Los1p, block spreading of heterochromatin by physical tethering of the HML locus to the NPC. These data demonstrate that physical tethering of genomic loci to the NPC controls their epigenetic activity. Genome-wide analysis of DNA-binding patterns for 14 transport proteins of the nuclear pore reveals that different NPC components bind different subsets of genes and that there is a relocalization of genes to the NPC upon transcriptional induction [215, 216].

In order to equalize the levels of X-chromosome-specific gene products in the cell to those present in females, *Drosophila* males activate a mechanisms called dosage compensation [217]. This mechanism involves the male-spectrum lethal (MSL) multimeric complex that hyperactivates the X chromosome by acetylating chromatin [218]. Components of the NPC were shown to be required for the correct localization of the dosage compensation complex (MSL), confirming that a subnuclear position has an active role in determining optimal gene expression levels of sex chromosomes [219]. Some evidence supports the idea that the NPC plays a role in faciliting RNA export. Indeed the association between active genes and components of the NPC is mediated by nascent RNA, because it is lost upon treatment with RNase [215]. Interestingly, anchoring to the NPC also requires the 3′-untranslated region (3′-UTR) of genes and appears to be crucial for triggering gene activation [220]. Thus, the association of genes with the NPC may play a key role in transcriptional regulation, possibly by topologically constraining repressed DNA segments or by facilitating the transcriptional activation and/or RNA export. However, the association of active genes/domains with the NPC may not be the rule in all cases [214]. Chromosome-wide data have estabilished that only 40% of genes interact constitutively through their promoter or intergenic region with the nuclear basket. This interaction is independent of transcription. Thus, it has been proposed that the connection between promoter and the NPC would be an early event involving gene activation.

In the budding yeast *Saccharomyces cerevisiae*, telomeres are associated with the nuclear periphery via the NPC [221, 222]. Components of the NPC are also required for full repression of the silent mating type loci [221, 223]. In yeast, telomere clustering is often accompanied by anchoring at the nuclear envelope, thus contributing to the global positioning of interphase chromosomes. The clustering of telomeres in budding yeast contributes to the repression of subtelomeric chromatin, conferring a telomere position effect (TPE), which resembles the PEV nucleated by centromeric repeats in flies [221, 223–226].

It remains to be determined which are the factors that tether the repressed loci to the nuclear periphery. In many cases, components of transcriptional silent chromatin are responsible for the positioning to the nuclear periphery. This is the case for yeast telomeres that are driven to the periphery by the telomere-associated proteins yKu and Sir4p, which also mediate telomere-telomere interactions [227]. As mentioned earlier, in *Drosophila* cells, the analysis of the distribution of *gypsy* insulator bodies indicates that they aggregate at a small number of nuclear locations near the lamina, the outer layer of the nuclear matrix [161] (Fig. 1C). Since the *gypsy* insulator complex is present in the nuclear matrix fraction, it has been suggested that components of the insulator interact with the nuclear matrix, contributing to the tethering of the looped DNA to the nuclear periphery [162].

Early observations identified RNA as an important component of the nuclear matrix [162, 228]. In yeast, mutations of components of the RNAi machinery affect the clustering of telomeres [229]. In *Drosophila*, it has been shown that the RNAi machinery influences the higher-order nuclear organization of the *gypsy* insulator and PRE function [230, 118], thereby affecting its ability to control gene expression [230]. The RNAi machinery and noncoding RNA could play a role in the control of nuclear architecture, although the precise mechanism of action is not clear.

Reprogramming

Currently, in the hope also to find therapeutical applications, one of the frontiers in biology is the comprehension of what defines stemness and pluripotency. The founding concepts inspiring these efforts return the scientific community to the classics of embryology. Experiments by J.B. Gurdon showed that at least two consecutive cycles of nuclear transplantations were necessary to allow cloned embryos from tadpole nuclei to reach more advanced stages of development [231]. These classic experiments anticipated investigation of the mechanisms by which terminally differentiated cells might regain totipoten-

cy and restart the life programme of a new individual or part of it.

During early mammalian development, cells undergo major epigenome 'reprogramming', involving the removal of epigenetic marks (DNA methylation and histone modification) followed by a *de novo* established set of marks [4, 6]. This happens specifically during germ cell differentiation and in the preimplantation embryo upon zygote formation. In particular, large-scale rearrangements involving both DNA and histone methylation and acetylation accompany the transition from fertilization to blastocyst formation [181]. These events are crucial for the success of the subsequent stages of development. During germ cell differentiation, in particular in the male germ line, profound changes take place in chromatin composition with the substitution of histone for protamins and polyamines. Upon fertilization, a series of regulated and largely obscure events occur so that the male genome rapidly regains the nucleosomal structure and the process of zygote formation is completed.

In somatic cloning, this process is bypassed or it does not take place properly. Indeed, in this case, the extent of erasure of epigenome components and marks is very limited [233]. Mammalian nuclei become more refractory to reprogramming with differentiation, whereas blastocyst-derived ES cells and totipotent cells in general support clone development at high efficiency [234]. It is likely that defects in epigenome reprogramming in cloned animals do not allow the competence and stability of the transcription programmes required for normal embryogenesis and development. Indeed, clones at various developmental stages and adults show dysregulation and persistence of expression of many donor-specific genes, and this might explain why cloned animals suffer from different and severe abnormalities [235, 236]. Although the transcriptome of nuclear-transfer-derived ES cells appears to be indistinguishable from that of fertilized-egg-derived ES cell lines, this programme might not be stable and/or reflect a real competence at the level of regulatory regions to transduce developmental signals in the correct manner [237]. In addition, the contribution of transcriptome analysis should also include the non-coding output of the genome [238, 239]. Specific combinations of transcription factors have been identified that enable reprogramming [240]. However, microarray analysis reveals that the real degree of reprogramming in these cells is limited to 50% of the transcriptome if compared to a normal ES cell. This suggests that a proper combination of transcription factors must also be integrated by changes in epigenome structure.

In general, complete erasure of the epigenetic memory and restoration of normal gene expression occurs only by passing through the germ line, as the offspring of cloned animals do not seem to present abnormalities [236]. Similarly, at least two rounds of 'reprogramming' of somatic nuclei appear to be required in order to allow efficient cloning [241, 242].

A key role in these processes appears to be played by the epigenome. Indeed, recent evidence in favour of a hyperdynamic chromatin structure in stem cells was described [232]. Regaining this condition may provide an opportunity window for signalling and transcription factors to establish a new programme in the cell. Transdetermination or reprogramming could be favoured by changes in the epigenome. It is likely that reduced activity of *writers* and *readers*, like PcG and DNMT proteins, might have an impact on reprogramming efficiency. Analysis of histone methylation in nuclei of quiescent (G_0) and proliferating B or T cells revealed that G_0 cells have markedly reduced levels of all H3 and H4 modifications [243]. Interestingly, nuclei from quiescent cells showed increased reprogramming potential [243]. Similar results were obtained in fibroblasts that were engineered to contain reduced levels of DNMT. They appear to perform better than fibroblast donors from normal blastocycts [244].

Recent results suggest that signalling pathways are essential for stem cell maintenance and reprogramming [245–247]. In *Drosophila*, the frequency of transdetermination is enhanced in PcG mutant flies [248]. Moreover, downregulation of PcG function, observed in transdeterminated cells, is directly controlled by the Jun amino-terminal kinase (JNK) signalling pathway [248]. During transdetermination, the prerequisite for the change in cell fate is a change in cell cycle regulation [249]. Indeed, different components of the PcG have been implicated in the regulation of cell proliferation [250–252], acting in either potent activators or repressors of the cell cycle. This suggests that the evolutionarity conserved PcG complexes could also play a role in resetting cell cycle regulation, resulting in transdetermination.

Another aspect concerns the changes in nuclear architecture that accompany reprogramming. Following on from Gurdon's seminal experiments, recent work by M. Mechali's laboratory showed that the efficiency of reprogramming of transplanted nuclei is dependent on the replicative potential induced by genome-wide reorganization and the average size of the replicons [253]. Surprisingly, replicon size rearrangement requires mitosis and chromosome condensation driven by topoisomerase II and is independent of the passage through S phase. Previous reports showed that in several organisms, the average size of matrix-attached DNA loops and replicons regulated

during early stages of development [254]. These results establish a relationship between reprogramming, mitosis, replicon size and DNA loop organization. Identification of the master regulators that control the mechanisms of replicon reorganization might indicate a target for reprogramming of differentiated cells.

Conclusion

Completion of the various genome projects has led to the identification of the primary, monodimensional structure of genetic information. This level of information appears to be insufficient for a complete understanding of how the networking among regulatory regions actually works. The contribution of additional 'coding' levels hidden in the three-dimensional structure of the chromosome and nuclear structures appears to be a fundamental aspect for the control of the quality and stability of genetic programmes. Fundamental efforts by bioinformaticians and systems biologists are still necessary to devise tools able to predict transcription factor target sites in the genome and determine how these target sequences actually act in the context of chromosome and nuclear organization. The analysis of epigenome structure may provide new databases that will integrate the primary sequence information. This includes genome-wide landscaping of histone modification, *in vivo* binding site identification of transcription and other chromosomal factors and three-dimensional analysis of *cis-trans* interactions by 3C technology. Another important aspect to be kept in mind is the fact that in mammals, almost 98% of the RNA output of the genome is 'non-coding'. This represents the 'dark side of the genome' that may well have an important role in regulation of the epigenome, and whose function still remains to be elucidated [238, 239]. What is clear is that each level of genome organization in the nucleus appears to play a role in the quality and quantity of gene regulation. Therefore, all efforts to 'reprogramme' cell fate must take into account all these levels of complexity.

At the end of the process, many epigenomes are formed by various components that modify chromosomal structures and contribute to the stability, heritability and also plasticity of transcription programmes. In this way, the history of an individual can be written into the genetic material. Thus, one can imagine the genome as a highly complex sheet music onto which many different flavours and colours can be added and transmitted beyond the DNA sequence. Most important, the harmony must last during the entire lifetime of an organism and in some cases beyond. Here comes the epigenome, similar to a music instrument that, depending on the composer, can play endlessly infinite tunes.

Acknowledgements. We apologize to all colleagues we could not cite due to space limitations. We thank A. Breiling for valuable comments on the manuscript. We are grateful to G. Oliva (Institute for High-Performance Computing and Networking, ICAR) for kind help in designing three dimensional images. V.O. is supported by Telethon Foundation, AIRC (Associazione Italiana Ricerca sul Cancro), Fondazione Compagnia San Paolo, EU-FP6 NoE The Epigenome, Ministery for University and Research (MIUR-FIRB).

1 Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. Science 293, 1074 – 80.
2 Turner, B.M. (2003) Memorable transcription. Nat. Cell Biol. 5, 390 – 3.
3 Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C. and Esteller, M. (2005) Epigenetic differences arise during the lifetime of monozygotic twins. Proc. Natl. Acad. Sci. USA 102, 10604 – 9.
4 Surani, M.A. (2001) Reprogramming of genome function through epigenetic inheritance. Nature 414, 122 – 8.
5 Jaenisch, R. and Bird, A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat. Genet. 33 Suppl, 245 – 54.
6 Rideout, W.M., 3rd, Eggan, K. and Jaenisch, R. (2001) Nuclear cloning and epigenetic reprogramming of the genome. Science 293, 1093 – 8.
7 Feinberg, A.P. and Tycko, B. (2004) The history of cancer epigenetics. Nat. Rev. Cancer 4, 143 – 53.
8 Goodell, M.A. (2003) Stem-cell "plasticity": befuddled by the muddle. Curr. Opin. Hematol. 10, 208 – 13.
9 Hsieh, J. and Gage, F.H. (2004) Epigenetic control of neural stem cell fate. Curr. Opin. Genet. Dev. 14, 461 – 9.
10 Pomerantz, J. and Blau, H.M. (2004) Nuclear reprogramming: a key to stem cell function in regenerative medicine. Nat. Cell Biol. 6, 810 – 6.
11 Klebes, A., Sustar, A., Kechris, K., Li, H., Schubiger, G. and Kornberg, T.B. (2005) Regulation of cellular plasticity in *Drosophila* imaginal disc cells by the Polycomb group, trithorax group and lama genes. Development 132, 3753 – 65.
12 Maurange, C., Lee, N. and Paro, R. (2006) Signaling meets chromatin during tissue regeneration in *Drosophila*. Curr. Opin. Genet. Dev. 16, 485 – 9.
13 Klose, R.J. and Bird, A.P. (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. 31, 89 – 97.
14 Takai, D. and Jones, P.A. (2003) The CpG island searcher: a new WWW resource. In Silico Biol. 3, 235 – 40.
15 Bird, A., Taggart, M., Frommer, M., Miller, O.J. and Macleod, D. (1985) A fraction of the mouse genome that is derived from islands of nonmethylated, CpG-rich DNA. Cell 40, 91 – 9.
16 Woodcock, D.M., Lawler, C.B., Linsenmeyer, M.E., Doherty, J.P. and Warren, W.D. (1997) Asymmetric methylation in the hypermethylated CpG promoter region of the human L1 retrotransposon. J. Biol. Chem. 272, 7810 – 6.
17 Bird, A. (2002) DNA methylation patterns and epigenetic memory. Genes Dev.. 16, 6 – 21.
18 Meehan, R.R. (2003) DNA methylation in animal development. Semin. Cell Dev. Biol. 14, 53 – 65.
19 Bird, A.P. and Wolffe, A.P. (1999) Methylation-induced repression – belts, braces, and chromatin. Cell 99, 451 – 4.
20 Siegfried, Z., Eden, S., Mendelsohn, M., Feng, X., Tsuberi, B.Z. and Cedar, H. (1999) DNA methylation represses transcription in vivo. Nat. Genet. 22, 203 – 6.

21 Feng, Y.Q., Desprat, R., Fu, H., Olivier, E., Lin, C.M., Lobell, A., Gowda, S.N., Aladjem, M.I. and Bouhassira, E.E. (2006) DNA methylation supports intrinsic epigenetic memory in mammalian cells. PLoS Genet. 2, e65.
22 Watt, F. and Molloy, P.L. (1988) Cytosine methylation prevents binding to DNA of a HeLa cell transcription factor required for optimal expression of the adenovirus major late promoter. Genes Dev. 2, 1136 – 43.
23 Lorincz, M.C., Dickerson, D.R., Schmitt, M. and Groudine, M. (2004) Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. Nat. Struct. Mol. Biol. 11, 1068 – 75.
24 Hendrich, B. and Bird, A. (1998) Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol. Cell Biol. 18, 6538 – 47.
25 Jones, P.L., Veenstra, G.J., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Strouboulis, J. and Wolffe, A.P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat. Genet. 19, 187 – 91.
26 Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N. and Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393, 386 – 9.
27 Ng, H.H., Zhang, Y., Hendrich, B., Johnson, C.A., Turner, B.M., Erdjument-Bromage, H., Tempst, P., Reinberg, D. and Bird, A. (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. Nat. Genet. 23, 58 – 61.
28 Sarraf, S.A. and Stancheva, I. (2004) Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. Mol. Cell 15, 595 – 605.
29 Wade, P.A., Gegonne, A., Jones, P.L., Ballestar, E., Aubry, F. and Wolffe, A.P. (1999) Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation. Nat. Genet. 23, 62 – 6.
30 Jackson, J.P., Lindroth, A.M., Cao, X. and Jacobsen, S.E. (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 416, 556 – 60.
31 Gehring, M., Huh, J.H., Hsieh, T.F., Penterman, J., Choi, Y., Harada, J.J., Goldberg, R.B. and Fischer, R.L. (2006) DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. Cell 124, 495 – 506.
32 Bell, A.C., West, A.G. and Felsenfeld, G. (1999) The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. Cell 98, 387 – 96.
33 Ohlsson, R., Renkawitz, R. and Lobanenkov, V. (2001) CTCF is a uniquely versatile transcription regulator linked to epigenetics and disease. Trends Genet., 17, 520 – 7.
34 Hark, A.T., Schoenherr, C.J., Katz, D.J., Ingram, R.S., Levorse, J.M. and Tilghman, S.M. (2000) CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. Nature 405, 486 – 9.
35 Colot, V. and Rossignol, J.L. (1999) Eukaryotic DNA methylation as an evolutionary device. Bioessays 21, 402 – 11.
36 Tweedie, S., Ng, H.H., Barlow, A.L., Turner, B.M., Hendrich, B. and Bird, A. (1999) Vestiges of a DNA methylation system in *Drosophila melanogaster*? Nat. Genet. 23, 389 – 90.
37 Marhold, J., Zbylut, M., Lankenau, D.H., Li, M., Gerlich, D., Ballestar, E., Mechler, B.M. and Lyko, F. (2002) Stage-specific chromosomal association of *Drosophila* dMBD2/3 during genome activation. Chromosoma 111, 13 – 21.
38 Marhold, J., Kramer, K., Kremmer, E. and Lyko, F. (2004) The *Drosophila* MBD2/3 protein mediates interactions between the MI-2 chromatin complex and CpT/A-methylated DNA. Development 131, 6033 – 9.
39 Luger, K. (2003) Structure and dynamic behavior of nucleosomes. Curr. Opin. Genet. Dev. 13, 127 – 35.
40 Khorasanizadeh, S. (2004) The nucleosome: from genomic organization to genomic regulation. Cell 116, 259 – 72.
41 Grunstein, M. (1997) Histone acetylation in chromatin structure and transcription. Nature 389, 349 – 52.
42 Sterner, D.E. and Berger, S.L. (2000) Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. 64, 435 – 59.
43 Richards, E.J. and Elgin, S.C. (2002) Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. Cell 108, 489 – 500.
44 Zhang, Y. and Reinberg, D. (2001) Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. Genes Dev. 15, 2343 – 60.
45 Nowak, S.J. and Corces, V.G. (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet. 20, 214 – 20.
46 Davie, J.R. and Murphy, L.C. (1990) Level of ubiquitinated histone H2B in chromatin is coupled to ongoing transcription. Biochemistry 29, 4752 – 7.
47 Nathan, D., Sterner, D.E. and Berger, S.L. (2003) Histone modifications: now summoning sumoylation. Proc. Natl. Acad. Sci. USA 100, 13118 – 20.
48 Wondrak, G.T., Cervantes-Laurean, D., Jacobson, E.L. and Jacobson, M.K. (2000) Histone carbonylation in vivo and in vitro. Biochem. J. 351, 769 – 77.
49 Huletsky, A., de Murcia, G., Muller, S., Hengartner, M., Menard, L., Lamarre, D. and Poirier, G.G. (1989) The effect of poly(ADP-ribosyl)ation on native and H1-depleted chromatin: a role of poly(ADP-ribosyl)ation on core nucleosome structure. J. Biol. Chem. 264, 8878 – 86.
50 Poirier, G.G., de Murcia, G., Jongstra-Bilen, J., Niedergang, C. and Mandel, P. (1982) Poly(ADP-ribosyl)ation of polynucleosomes causes relaxation of chromatin structure. Proc. Natl. Acad. Sci. USA 79, 3423 – 7.
51 Felsenfeld, G. and Groudine, M. (2003) Controlling the double helix. Nature 421, 448 – 53.
52 Turner, B.M. (2002) Cellular memory and the histone code. Cell 111, 285 – 91.
53 Cao, R. and Zhang, Y. (2004) The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr. Opin. Genet. Dev. 14, 155 – 64.
54 Jeppesen, P. and Turner, B.M. (1993) The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. Cell 74, 281 – 9.
55 Turner, B.M., Birley, A.J. and Lavender, J. (1992) Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. Cell 69, 375 – 84.
56 Strahl, B.D. and Allis, C.D. (2000) The language of covalent histone modifications. Nature 403, 41 – 5.
57 Henikoff, S. (2005) Histone modifications: combinatorial complexity or cumulative simplicity? Proc. Natl. Acad. Sci. USA 102, 5308 – 9.
58 Dion, M.F., Altschuler, S.J., Wu, L.F. and Rando, O.J. (2005) Genomic characterization reveals a simple histone H4 acetylation code. Proc. Natl. Acad. Sci. USA 102, 5501 – 6.
59 Jacobs, S.A., Taverna, S.D., Zhang, Y., Briggs, S.D., Li, J., Eissenberg, J.C., Allis, C.D. and Khorasanizadeh, S. (2001) Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. EMBO J. 20, 5232 – 41.
60 Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. and Jenuwein, T. (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410, 116 – 20.
61 Mutskov, V. and Felsenfeld, G. (2004) Silencing of transgene transcription precedes methylation of promoter DNA and histone H3 lysine 9. EMBO J. 23, 138 – 49.
62 Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D. and Grewal, S.I. (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292, 110 – 3.
63 Papp, B. and Muller, J. (2006) Histone trimethylation and the

maintenance of transcriptional ON and OFF states by trxG and PcG proteins. Genes Dev. 20, 2041 – 54.
64 Stewart, M.D., Li, J. and Wong, J. (2005) Relationship between histone H3 lysine 9 methylation, transcription repression, and heterochromatin protein 1 recruitment. Mol. Cell Biol. 25, 2525 – 38.
65 Bernstein, B.E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D.K., Huebert, D.J., McMahon, S., Karlsson, E.K., Kulbokas, E.J., 3rd, Gingeras, T.R., Schreiber, S.L. and Lander, E.S. (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 120, 169 – 81.
66 Pokholok, D.K., Harbison, C.T., Levine, S., Cole, M., Hannett, N.M., Lee, T.I., Bell, G.W., Walker, K., Rolfe, P.A., Herbolsheimer, E., Zeitlinger, J., Lewitter, F., Gifford, D.K. and Young, R.A. (2005) Genome-wide map of nucleosome acetylation and methylation in yeast. Cell 122, 517 – 27.
67 Santos-Rosa, H., Schneider, R., Bannister, A.J., Sherriff, J., Bernstein, B.E., Emre, N.C., Schreiber, S.L., Mellor, J. and Kouzarides, T. (2002) Active genes are tri-methylated at K4 of histone H3. Nature 419, 407 – 11.
68 Schneider, R., Bannister, A.J., Myers, F.A., Thorne, A.W., Crane-Robinson, C. and Kouzarides, T. (2004) Histone H3 lysine 4 methylation patterns in higher eukaryotic genes. Nat. Cell Biol. 6, 73 – 7.
69 Schubeler, D., MacAlpine, D.M., Scalzo, D., Wirbelauer, C., Kooperberg, C., van Leeuwen, F., Gottschling, D.E., O'Neill, L.P., Turner, B.M., Delrow, J., Bell, S.P. and Groudine, M. (2004) The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. Genes Dev. 18, 1263 – 71.
70 Ng, H.H., Xu, R.M., Zhang, Y. and Struhl, K. (2002) Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. J. Biol. Chem. 277, 34655 – 7.
71 Sun, Z.W. and Allis, C.D. (2002) Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. Nature 418, 104 – 8.
72 Fischle, W., Wang, Y. and Allis, C.D. (2003) Binary switches and modification cassettes in histone biology and beyond. Nature 425, 475 – 9.
73 Mateescu, B., England, P., Halgand, F., Yaniv, M. and Muchardt, C. (2004) Tethering of HP1 proteins to chromatin is relieved by phosphoacetylation of histone H3. EMBO Rep. 5, 490 – 6.
74 Kurdistani, S.K., Tavazoie, S. and Grunstein, M. (2004) Mapping global histone acetylation patterns to gene expression. Cell 117, 721 – 33.
75 Bae, E., Calhoun, V.C., Levine, M., Lewis, E.B. and Drewell, R.A. (2002) Characterization of the intergenic RNA profile at abdominal-A and Abdominal-B in the *Drosophila* bithorax complex. Proc. Natl. Acad. Sci. USA 99, 16847 – 52.
76 Rank, G., Prestel, M. and Paro, R. (2002) Transcription through intergenic chromosomal memory elements of the Drosophila bithorax complex correlates with an epigenetic switch. Mol. Cell Biol. 22, 8026 – 34.
77 Sessa, L., Breiling, A., Lavorgna, G., Silvestri, L., Casari, G. and Orlando, V. (2006) Noncoding RNA synthesis and loss of Polycomb group repression accompanies the colinear activation of the human HOXA cluster. RNA. 13, 223 – 239.
78 Guccione, E., Martinato, F., Finocchiaro, G., Luzi, L., Tizzoni, L., Dall' Olio, V., Zardo, G., Nervi, C., Bernard, L. and Amati, B. (2006) Myc-binding-site recognition in the human genome is determined by chromatin context. Nat. Cell Biol. 8, 764 – 70.
79 Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thastrom, A., Field, Y., Moore, I.K., Wang, J.P. and Widom, J. (2006) A genomic code for nucleosome positioning. Nature 442, 772 – 8.
80 Gal-Yam, E.N., Jeong, S., Tanay, A., Egger, G., Lee, A.S. and Jones, P.A. (2006) Constitutive nucleosome depletion and ordered factor assembly at the GRP78 promoter revealed by single molecule footprinting. PLoS Genet 2, e160.
81 Mohd-Sarip, A., van der Knaap, J.A., Wyman, C., Kanaar, R., Schedl, P. and Verrijzer, C.P. (2006) Architecture of a polycomb nucleoprotein complex. Mol. Cell 24, 91 – 100.
82 Yamamoto, K. and Sonoda, M. (2003) Self-interaction of heterochromatin protein 1 is required for direct binding to histone methyltransferase, SUV39H1. Biochem. Biophys. Res. Commun. 301, 287 – 92.
83 Maison, C., Bailly, D., Peters, A.H., Quivy, J.P., Roche, D., Taddei, A., Lachner, M., Jenuwein, T. and Almouzni, G. (2002) Higher-order structure in pericentric heterochromatin involves a distinct pattern of histone modification and an RNA component. Nat. Genet. 30, 329 – 34.
84 Muchardt, C., Guilleme, M., Seeler, J.S., Trouche, D., Dejean, A. and Yaniv, M. (2002) Coordinated methyl and RNA binding is required for heterochromatin localization of mammalian HP1alpha. EMBO Rep. 3, 975 – 81.
85 Maison, C. and Almouzni, G. (2004) HP1 and the dynamics of heterochromatin maintenance. Nat. Rev. Mol. Cell Biol. 5, 296 – 304.
86 Perrini, B., Piacentini, L., Fanti, L., Altieri, F., Chichiarelli, S., Berloco, M., Turano, C., Ferraro, A. and Pimpinelli, S. (2004) HP1 controls telomere capping, telomere elongation, and telomere silencing by two different mechanisms in *Drosophila*. Mol. Cell 15, 467 – 76.
87 Saveliev, A., Everett, C., Sharpe, T., Webster, Z. and Festenstein, R. (2003) DNA triplet repeats mediate heterochromatin-protein-1-sensitive variegated gene silencing. Nature 422, 909 – 13.
88 Nielsen, S.J., Schneider, R., Bauer, U.M., Bannister, A.J., Morrison, A., O'Carroll, D., Firestein, R., Cleary, M., Jenuwein, T., Herrera, R.E. and Kouzarides, T. (2001) Rb targets histone H3 methylation and HP1 to promoters. Nature 412, 561 – 5.
89 Pak, D.T., Pflumm, M., Chesnokov, I., Huang, D.W., Kellum, R., Marr, J., Romanowski, P. and Botchan, M.R. (1997) Association of the origin recognition complex with heterochromatin and HP1 in higher eukaryotes. Cell 91, 311 – 23.
90 Piacentini, L., Fanti, L., Berloco, M., Perrini, B. and Pimpinelli, S. (2003) Heterochromatin protein 1 (HP1) is associated with induced gene expression in *Drosophila* euchromatin. J. Cell Biol. 161, 707 – 14.
91 Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M.E., Borchers, C.H., Tempst, P. and Zhang, Y. (2006) Histone demethylation by a family of JmjC domain-containing proteins. Nature 439, 811 – 6.
92 Grewal, S.I. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. Science 301, 798 – 802.
93 Buhler, M., Verdel, A. and Moazed, D. (2006) Tethering RITS to a nascent transcript initiates RNAi- and heterochromatin-dependent gene silencing. Cell 125, 873 – 86.
94 Zofall, M. and Grewal, S.I. (2006) Swi6/HP1 recruits a JmjC domain protein to facilitate transcription of heterochromatic repeats. Mol. Cell 22, 681 – 92.
95 Kim, M.Y., Zhang, T. and Kraus, W.L. (2005) Poly(ADP-ribosyl)ation by PARP-1: 'PAR-laying' NAD+ into a nuclear signal. Genes Dev. 19, 1951 – 67.
96 Kraus, W.L. and Lis, J.T. (2003) PARP goes transcription. Cell 113, 677 – 83.
97 Tulin, A. and Spradling, A. (2003) Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at *Drosophila* puff loci. Science 299, 560 – 2.
98 Kim, M.Y., Mauro, S., Gevry, N., Lis, J.T. and Kraus, W.L. (2004) NAD+-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. Cell 119, 803 – 14.
99 Bantignies, F. and Cavalli, G. (2006) Cellular memory and dynamic regulation of polycomb group proteins. Curr. Opin. Cell Biol. 18, 275 – 83.
100 Jacobs, J.J. and van Lohuizen, M. (2002) Polycomb repression:

from cellular memory to cellular proliferation and cancer. Biochim. Biophys. Acta 1602, 151 – 61.
101 Lessard, J. and Sauvageau, G. (2003) Polycomb group genes as epigenetic regulators of normal and leukemic hemopoiesis. Exp. Hematol. 31, 567 – 85.
102 Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R.S. and Zhang, Y. (2002) Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science 298, 1039 – 43.
103 Czermin, B., Melfi, R., McCabe, D., Seitz, V., Imhof, A. and Pirrotta, V. (2002) *Drosophila* enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell 111, 185 – 96.
104 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.
105 Cavalli, G. and Paro, R. (1998) The *Drosophila* Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. Cell 93, 505 – 18.
106 Ringrose, L. and Paro, R. (2004) Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu: Rev: Genet: 38, 413 – 43.
107 Ringrose, L., Rehmsmeier, M., Dura, J.M. and Paro, R. (2003) Genome-wide prediction of Polycomb/Trithorax response elements in *Drosophila melanogaster*. Dev. Cell 5, 759 – 71.
108 Orlando, V. (2003) Polycomb, epigenomes, and control of cell identity. Cell 112, 599 – 606.
109 Breiling, A., Turner, B.M., Bianchi, M.E. and Orlando, V. (2001) General transcription factors bind promoters repressed by Polycomb group proteins. Nature 412, 651 – 5.
110 Orlando, V., Jane, E.P., Chinwalla, V., Harte, P.J. and Paro, R. (1998) Binding of trithorax and Polycomb proteins to the bithorax complex: dynamic changes during early *Drosophila* embryogenesis. EMBO J. 17, 5141 – 50.
111 Wang, L., Brown, J.L., Cao, R., Zhang, Y., Kassis, J.A. and Jones, R.S. (2004) Hierarchical recruitment of polycomb group silencing complexes. Mol. Cell 14, 637 – 46.
112 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., Bell, G.W., Otte, A.P., Vidal, M., Gifford, D.K., Young, R.A. and Jaenisch, R. (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 441, 349 – 53.
113 Schwartz, Y.B., Kahn, T.G., Nix, D.A., Li, X.Y., Bourgon, R., Biggin, M. and Pirrotta, V. (2006) Genome-wide analysis of Polycomb targets in *Drosophila melanogaster*. Nat. Genet. 38, 700 – 5.
114 Buchenau, P., Hodgson, J., Strutt, H. and Arndt-Jovin, D.J. (1998) The distribution of polycomb-group proteins during cell division and development in *Drosophila* embryos: impact on models for silencing. J. Cell Biol. 141, 469 – 81.
115 Ficz, G., Heintzmann, R. and Arndt-Jovin, D.J. (2005) Polycomb group protein complexes exchange rapidly in living *Drosophila*. Development 132, 3963 – 76.
116 Lupo, R., Breiling, A., Bianchi, M.E. and Orlando, V. (2001) *Drosophila* chromosome condensation proteins Topoisomerase II and Barren colocalize with Polycomb and maintain Fab-7 PRE silencing. Mol. Cell 7, 127 – 36.
117 Saurin, A.J., Shao, Z., Erdjument-Bromage, H., Tempst, P. and Kingston, R.E. (2001) A *Drosophila* Polycomb group complex includes Zeste and dTAFII proteins. Nature 412, 655 – 60.
118 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 – 71.
119 Vire, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., Morey, L., Van Eynde, A., Bernard, D., Vanderwinden, J.M., Bollen, M., Esteller, M., Di Croce, L., de Launoit, Y. and Fuks, F. (2006) The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439, 871 – 4.
120 Hernandez-Munoz, I., Taghavi, P., Kuijl, C., Neefjes, J. and van Lohuizen, M. (2005) Association of BMI1 with polycomb bodies is dynamic and requires PRC2/EZH2 and the maintenance DNA methyltransferase DNMT1. Mol. Cell Biol. 25, 11047 – 58.
121 Sparmann, A. and van Lohuizen, M. (2006) Polycomb silencers control cell fate, development and cancer. Nat. Rev. Cancer 6, 846 – 56.
122 Cha, T.L., Zhou, B.P., Xia, W., Wu, Y., Yang, C.C., Chen, C.T., Ping, B., Otte, A.P. and Hung, M.C. (2005) Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. Science 310, 306 – 10.
123 Su, I.H., Dobenecker, M.W., Dickinson, E., Oser, M., Basavaraj, A., Marqueron, R., Viale, A., Reinberg, D., Wulfing, C. and Tarakhovsky, A. (2005) Polycomb group protein ezh2 controls actin polymerization and cell signaling. Cell 121, 425 – 36.
124 Flanagan, J.F., Mi, L.Z., Chruszcz, M., Cymborowski, M., Clines, K.L., Kim, Y., Minor, W., Rastinejad, F. and Khorasanizadeh, S. (2005) Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature 438, 1181 – 5.
125 Li, H., Ilin, S., Wang, W., Duncan, E.M., Wysocka, J., Allis, C.D. and Patel, D.J. (2006) Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. Nature 442, 91 – 5.
126 Pena, P.V., Davrazou, F., Shi, X., Walter, K.L., Verkhusha, V.V., Gozani, O., Zhao, R. and Kutateladze, T.G. (2006) Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. Nature 442, 100 – 3.
127 Pray-Grant, M.G., Daniel, J.A., Schieltz, D., Yates, J.R., 3rd and Grant, P.A. (2005) Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. Nature 433, 434 – 8.
128 Shi, X., Hong, T., Walter, K.L., Ewalt, M., Michishita, E., Hung, T., Carney, D., Pena, P., Lan, F., Kaadige, M.R., Lacoste, N., Cayrou, C., Davrazou, F., Saha, A., Cairns, B.R., Ayer, D.E., Kutateladze, T.G., Shi, Y., Cote, J., Chua, K.F. and Gozani, O. (2006) ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 442, 96 – 9.
129 Sims, R.J., 3rd, Chen, C.F., Santos-Rosa, H., Kouzarides, T., Patel, S.S. and Reinberg, D. (2005) Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J. Biol. Chem. 280, 41789 – 92.
130 Wysocka, J., Swigut, T., Xiao, H., Milne, T.A., Kwon, S.Y., Landry, J., Kauer, M., Tackett, A.J., Chait, B.T., Badenhorst, P., Wu, C. and Allis, C.D. (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. Nature 442, 86 – 90.
131 Bienz, M. (2006) The PHD finger, a nuclear protein-interaction domain. Trends Biochem. Sci. 31, 35 – 40.
132 Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K. and Zhou, M.M. (1999) Structure and ligand of a histone acetyltransferase bromodomain. Nature 399, 491 – 6.
133 Ragvin, A., Valvatne, H., Erdal, S., Arskog, V., Tufteland, K.R., Breen, K., AM, O.Y., Eberharter, A., Gibson, T.J., Becker, P.B. and Aasland, R. (2004) Nucleosome binding by the bromodomain and PHD finger of the transcriptional cofactor p300. J. Mol. Biol. 337, 773 – 88.
134 Zhou, Y. and Grummt, I. (2005) The PHD finger/bromodomain of NoRC interacts with acetylated histone H4K16 and is sufficient for rDNA silencing. Curr. Biol. 15, 1434 – 8.
135 Sarma, K. and Reinberg, D. (2005) Histone variants meet their match. Nat. Rev. Mol. Cell Biol. 6, 139 – 49.
136 Bosch, A. and Suau, P. (1995) Changes in core histone variant composition in differentiating neurons: the roles of differential turnover and synthesis rates. Eur J. Cell Biol. 68, 220 – 5.
137 Fan, J.Y., Rangasamy, D., Luger, K. and Tremethick, D.J.

(2004) H2A.Z alters the nucleosome surface to promote HP1alpha-mediated chromatin fiber folding. Mol. Cell 16, 655 – 61.
138 Rangasamy, D., Berven, L., Ridgway, P. and Tremethick, D.J. (2003) Pericentric heterochromatin becomes enriched with H2A.Z during early mammalian development. EMBO J. 22, 1599 – 607.
139 Wunsch, A.M., Reinhardt, K. and Lough, J. (1991) Normal transitions in synthesis of replacement histones H2A.Z and H3.3 during differentiation of dystrophic myotube cells: a brief note. Mech. Ageing Dev. 59, 299 – 305.
140 Ahmad, K. and Henikoff, S. (2002) Epigenetic consequences of nucleosome dynamics. Cell 111, 281 – 4.
141 Akhmanova, A.S., Bindels, P.C., Xu, J., Miedema, K., Kremer, H. and Hennig, W. (1995) Structure and expression of histone H3.3 genes in *Drosophila melanogaster* and *Drosophila hydei*. Genome 38, 586 – 600.
142 Ahmad, K. and Henikoff, S. (2002) The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. Mol. Cell 9, 1191 – 200.
143 Chow, C.M., Georgiou, A., Szutorisz, H., Maia e Silva, A., Pombo, A., Barahona, I., Dargelos, E., Canzonetta, C. and Dillon, N. (2005) Variant histone H3.3 marks promoters of transcriptionally active genes during mammalian cell division. EMBO Rep. 6, 354 – 60.
144 Janicki, S.M., Tsukamoto, T., Salghetti, S.E., Tansey, W.P., Sachidanandam, R., Prasanth, K.V., Ried, T., Shav-Tal, Y., Bertrand, E., Singer, R.H. and Spector, D.L. (2004) From silencing to gene expression: real-time analysis in single cells. Cell 116, 683 – 98.
145 Schwartz, B.E. and Ahmad, K. (2005) Transcriptional activation triggers deposition and removal of the histone variant H3.3. Genes Dev. 19, 804 – 14.
146 Mito, Y., Henikoff, J.G. and Henikoff, S. (2005) Genome-scale profiling of histone H3.3 replacement patterns. Nat. Genet. 37, 1090 – 7.
147 McKittrick, E., Gafken, P.R., Ahmad, K. and Henikoff, S. (2004) Histone H3.3 is enriched in covalent modifications associated with active chromatin. Proc. Natl. Acad. Sci. USA 101, 1525 – 30.
148 Allis, C.D., Richman, R., Gorovsky, M.A., Ziegler, Y.S., Touchstone, B., Bradley, W.A. and Cook, R.G. (1986) hv1 is an evolutionarily conserved H2A variant that is preferentially associated with active genes. J. Biol. Chem. 261, 1941 – 8.
149 Clarkson, M.J., Wells, J.R., Gibson, F., Saint, R. and Tremethick, D.J. (1999) Regions of variant histone His2AvD required for *Drosophila* development. Nature 399, 694 – 7.
150 Faast, R., Thonglairoam, V., Schulz, T.C., Beall, J., Wells, J.R., Taylor, H., Matthaei, K., Rathjen, P.D., Tremethick, D.J. and Lyons, I. (2001) Histone variant H2A.Z is required for early mammalian development. Curr. Biol. 11, 1183 – 7.
151 Meneghini, M.D., Wu, M. and Madhani, H.D. (2003) Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell 112, 725 – 36.
152 Raisner, R.M., Hartley, P.D., Meneghini, M.D., Bao, M.Z., Liu, C.L., Schreiber, S.L., Rando, O.J. and Madhani, H.D. (2005) Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. Cell 123, 233 – 48.
153 Li, B., Pattenden, S.G., Lee, D., Gutierrez, J., Chen, J., Seidel, C., Gerton, J. and Workman, J.L. (2005) Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. Proc. Natl. Acad. Sci. USA 102, 18385 – 90.
154 Zhang, H., Roberts, D.N. and Cairns, B.R. (2005) Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell 123, 219 – 31.
155 Krogan, N.J., Keogh, M.C., Datta, N., Sawa, C., Ryan, O.W., Ding, H., Haw, R.A., Pootoolal, J., Tong, A., Canadien, V., Richards, D.P., Wu, X., Emili, A., Hughes, T.R., Buratowski, S. and Greenblatt, J.F. (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol. Cell 12, 1565 – 76.
156 Rangasamy, D., Greaves, I. and Tremethick, D.J. (2004) RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. Nat. Struct. Mol. Biol. 11, 650 – 5.
157 Chambeyron, S. and Bickmore, W.A. (2004) Chromatin decondensation and nuclear reorganization of the HoxB locus upon induction of transcription. Genes Dev. 18, 1119 – 30.
158 Ishii, K., Arib, G., Lin, C., Van Houwe, G. and Laemmli, U.K. (2002) Chromatin boundaries in budding yeast: the nuclear pore connection. Cell 109, 551 – 62.
159 West, A.G. and Fraser, P. (2005) Remote control of gene transcription. Hum. Mol. Genet. 14 Spec. No. 1, R101 – R111.
160 Kuhn, E.J. and Geyer, P.K. (2003) Genomic insulators: connecting properties to mechanism. Curr. Opin. Cell Biol. 15, 259 – 65.
161 Gerasimova, T.I., Byrd, K. and Corces, V.G. (2000) A chromatin insulator determines the nuclear localization of DNA. Mol. Cell 6, 1025 – 35.
162 Byrd, K. and Corces, V.G. (2003) Visualization of chromatin domains created by the gypsy insulator of *Drosophila*. J. Cell Biol. 162, 565 – 74.
163 Blanton, J., Gaszner, M. and Schedl, P. (2003) Protein:protein interactions and the pairing of boundary elements in vivo. Genes Dev. 17, 664 – 75.
164 Noma, K., Cam, H.P., Maraia, R.J. and Grewal, S.I. (2006) A role for TFIIIC transcription factor complex in genome organization. Cell 125, 859 – 72.
165 Kropotov, A.V. and Tomilin, N.V. (1996) Evidence for a regulatory protein complex on RNA polymerase III promoter of human retroposons of Alu family. Genetica 98, 223 – 33.
166 Cai, H.N. and Shen, P. (2001) Effects of cis arrangement of chromatin insulators on enhancer-blocking activity. Science 291, 493 – 5.
167 Comet, I., Savitskaya, E., Schuettengruber, B., Negre, N., Lavrov, S., Parshikov, A., Juge, F., Gracheva, E., Georgiev, P. and Cavalli, G. (2006) PRE-mediated bypass of two Su(Hw) insulators targets PcG proteins to a downstream promoter. Dev. Cell 11, 117 – 24.
168 Ronshaugen, M. and Levine, M. (2004) Visualization of trans-homolog enhancer-promoter interactions at the Abd-B Hox locus in the *Drosophila* embryo. Dev. Cell 7, 925 – 32.
169 Cleard, F., Moshkin, Y., Karch, F. and Maeda, R.K. (2006) Probing long-distance regulatory interactions in the *Drosophila melanogaster* bithorax complex using Dam identification. Nat. Genet. 38, 931 – 5.
170 Gorski, S.A., Dundr, M. and Misteli, T. (2006) The road much traveled: trafficking in the cell nucleus. Curr. Opin. Cell Biol. 18, 284 – 90.
171 Bantignies, F., Grimaud, C., Lavrov, S., Gabut, M. and Cavalli, G. (2003) Inheritance of Polycomb-dependent chromosomal interactions in *Drosophila*. Genes Dev. 17, 2406 – 20.
172 Sigrist, C.J. and Pirrotta, V. (1997) Chromatin insulator elements block the silencing of a target gene by the *Drosophila* polycomb response element (PRE) but allow trans interactions between PREs on different chromosomes. Genetics 147, 209 – 21.
173 Pirrotta, V. (1997) Chromatin-silencing mechanisms in *Drosophila* maintain patterns of gene expression. Trends Genet. 13, 314 – 8.
174 Vazquez, J., Muller, M., Pirrotta, V. and Sedat, J.W. (2006) The Mcp element mediates stable long-range chromosome-chromosome interactions in *Drosophila*. Mol. Biol. Cell 17, 2158 – 65.
175 Barna, M., Merghoub, T., Costoya, J.A., Ruggero, D., Branford, M., Bergia, A., Samori, B. and Pandolfi, P.P. (2002) Plzf mediates transcriptional repression of HoxD gene expression through chromatin remodeling. Dev. Cell 3, 499 – 510.
176 de Laat, W. and Grosveld, F. (2003) Spatial organization of

gene expression: the active chromatin hub. Chromosome Res. 11, 447 – 59.
177 Tolhuis, B., Palstra, R.J., Splinter, E., Grosveld, F. and de Laat, W. (2002) Looping and interaction between hypersensitive sites in the active beta-globin locus. Mol. Cell 10, 1453 – 65.
178 Vakoc, C.R., Letting, D.L., Gheldof, N., Sawado, T., Bender, M.A., Groudine, M., Weiss, M.J., Dekker, J. and Blobel, G.A. (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. Mol. Cell 17, 453 – 62.
179 Drissen, R., Palstra, R.J., Gillemans, N., Splinter, E., Grosveld, F., Philipsen, S. and de Laat, W. (2004) The active spatial organization of the beta-globin locus requires the transcription factor EKLF. Genes Dev. 18, 2485 – 90.
180 Splinter, E., Heath, H., Kooren, J., Palstra, R.J., Klous, P., Grosveld, F., Galjart, N. and de Laat, W. (2006) CTCF mediates long-range chromatin looping and local histone modification in the beta-globin locus. Genes Dev. 20, 2349 – 54.
181 Morgan, H.D., Santos, F., Green, K., Dean, W. and Reik, W. (2005) Epigenetic reprogramming in mammals. Hum. Mol. Genet. 14 Spec. No. 1, R47 – R58.
182 Verona, R.I., Mann, M.R. and Bartolomei, M.S. (2003) Genomic imprinting: intricacies of epigenetic regulation in clusters. Annu. Rev. Cell Dev. Biol. 19, 237 – 59.
183 Dekker, J. (2006) The three 'C' s of chromosome conformation capture: controls, controls, controls. Nat Methods 3, 17 – 21.
184 Murrell, A., Heeson, S. and Reik, W. (2004) Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. Nat. Genet. 36, 889 – 93.
185 Burke, L.J., Zhang, R., Bartkuhn, M., Tiwari, V.K., Tavoosidana, G., Kurukuti, S., Weth, C., Leers, J., Galjart, N., Ohlsson, R. and Renkawitz, R. (2005) CTCF binding and higher order chromatin structure of the H19 locus are maintained in mitotic chromatin. EMBO J. 24, 3291 – 300.
186 Kurukuti, S., Tiwari, V.K., Tavoosidana, G., Pugacheva, E., Murrell, A., Zhao, Z., Lobanenkov, V., Reik, W. and Ohlsson, R. (2006) CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. Proc. Natl. Acad. Sci. USA 103, 10684 – 9.
187 Fedoriw, A.M., Stein, P., Svoboda, P., Schultz, R.M. and Bartolomei, M.S. (2004) Transgenic RNAi reveals essential function for CTCF in H19 gene imprinting. Science 303, 238 – 40.
188 Rand, E., Ben-Porath, I., Keshet, I. and Cedar, H. (2004) CTCF elements direct allele-specific undermethylation at the imprinted H19 locus. Curr. Biol. 14, 1007 – 12.
189 Ling, J.Q., Li, T., Hu, J.F., Vu, T.H., Chen, H.L., Qiu, X.W., Cherry, A.M. and Hoffman, A.R. (2006) CTCF mediates interchromosomal colocalization between Igf2/H19 and Wsb1/Nf1. Science 312, 269 – 72.
190 Horike, S., Cai, S., Miyano, M., Cheng, J.F. and Kohwi-Shigematsu, T. (2005) Loss of silent-chromatin looping and impaired imprinting of DLX5 in Rett syndrome. Nat. Genet. 37, 31 – 40.
191 Dickinson, L.A., Joh, T., Kohwi, Y. and Kohwi-Shigematsu, T. (1992) A tissue-specific MAR/SAR DNA-binding protein with unusual binding site recognition. Cell 70, 631 – 45.
192 Yasui, D., Miyano, M., Cai, S., Varga-Weisz, P. and Kohwi-Shigematsu, T. (2002) SATB1 targets chromatin remodelling to regulate genes over long distances. Nature 419, 641 – 5.
193 Cai, S., Lee, C.C. and Kohwi-Shigematsu, T. (2006) SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. Nat. Genet. 38, 1278 – 88.
194 Cremer, T., Kupper, K., Dietzel, S. and Fakan, S. (2004) Higher order chromatin architecture in the cell nucleus: on the way from structure to function. Biol Cell 96, 555 – 67.
195 Misteli, T. (2004) Spatial positioning; a new dimension in genome function. Cell 119, 153 – 6.
196 Sproul, D., Gilbert, N. and Bickmore, W.A. (2005) The role of chromatin structure in regulating the expression of clustered genes. Nat Rev Genet 6, 775 – 81.
197 Cremer, T., Cremer, M., Dietzel, S., Muller, S., Solovei, I. and Fakan, S. (2006) Chromosome territories – a functional nuclear landscape. Curr. Opin. Cell Biol. 18, 307 – 16.
198 Cremer, T. and Cremer, C. (2001) Chromosome territories, nuclear architecture and gene regulation in mammalian cells. Nat Rev Genet 2, 292 – 301.
199 Mahy, N.L., Perry, P.E., Gilchrist, S., Baldock, R.A. and Bickmore, W.A. (2002) Spatial organization of active and inactive genes and noncoding DNA within chromosome territories. J. Cell Biol. 157, 579 – 89.
200 Brown, K.E., Guest, S.S., Smale, S.T., Hahm, K., Merkenschlager, M. and Fisher, A.G. (1997) Association of transcriptionally silent genes with Ikaros complexes at centromeric heterochromatin. Cell 91, 845 – 54.
201 Brown, K.E., Amoils, S., Horn, J.M., Buckle, V.J., Higgs, D.R., Merkenschlager, M. and Fisher, A.G. (2001) Expression of alpha- and beta-globin genes occurs within different nuclear domains in haemopoietic cells. Nat. Cell Biol. 3, 602 – 6.
202 Volpi, E.V., Chevret, E., Jones, T., Vatcheva, R., Williamson, J., Beck, S., Campbell, R.D., Goldsworthy, M., Powis, S.H., Ragoussis, J., Trowsdale, J. and Sheer, D. (2000) Large-scale chromatin organization of the major histocompatibility complex and other regions of human chromosome 6 and its response to interferon in interphase nuclei. J. Cell Sci. 113, 1565 – 76.
203 Williams, R.R., Broad, S., Sheer, D. and Ragoussis, J. (2002) Subchromosomal positioning of the epidermal differentiation complex (EDC) in keratinocyte and lymphoblast interphase nuclei. Exp. Cell Res. 272, 163 – 75.
204 Tumbar, T., Sudlow, G. and Belmont, A.S. (1999) Large-scale chromatin unfolding and remodeling induced by VP16 acidic activation domain. J. Cell Biol. 145, 1341 – 54.
205 Verschure, P.J., van der Kraan, I., de Leeuw, W., van der Vlag, J., Carpenter, A.E., Belmont, A.S. and van Driel, R. (2005) In vivo HP1 targeting causes large-scale chromatin condensation and enhanced histone lysine methylation. Mol. Cell Biol. 25, 4552 – 64.
206 Chambeyron, S., Da Silva, N.R., Lawson, K.A. and Bickmore, W.A. (2005) Nuclear re-organisation of the Hoxb complex during mouse embryonic development. Development 132, 2215 – 23.
207 Gilbert, N., Boyle, S., Fiegler, H., Woodfine, K., Carter, N.P. and Bickmore, W.A. (2004) Chromatin architecture of the human genome: gene-rich domains are enriched in open chromatin fibers. Cell 118, 555 – 66.
208 Osborne, C.S., Chakalova, L., Brown, K.E., Carter, D., Horton, A., Debrand, E., Goyenechea, B., Mitchell, J.A., Lopes, S., Reik, W. and Fraser, P. (2004) Active genes dynamically colocalize to shared sites of ongoing transcription. Nat. Genet. 36, 1065 – 71.
209 Dror, V. and Winston, F. (2004) The Swi/Snf chromatin remodeling complex is required for ribosomal DNA and telomeric silencing in *Saccharomyces cerevisiae*. Mol. Cell Biol. 24, 8227 – 35.
210 Spilianakis, C.G., Lalioti, M.D., Town, T., Lee, G.R. and Flavell, R.A. (2005) Interchromosomal associations between alternatively expressed loci. Nature 435, 637 – 45.
211 Simonis, M., Klous, P., Splinter, E., Moshkin, Y., Willemsen, R., de Wit, E., van Steensel, B. and de Laat, W. (2006) Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation capture-on-chip (4C). Nat. Genet. 38, 1348 – 54.
212 Marshall, W.F., Dernburg, A.F., Harmon, B., Agard, D.A. and Sedat, J.W. (1996) Specific interactions of chromatin with the nuclear envelope: positional determination within the nucleus in *Drosophila melanogaster*. Mol. Biol. Cell 7, 825 – 42.

213 Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M. and van Steensel, B. (2006) Characterization of the *Drosophila melanogaster* genome at the nuclear lamina. Nat. Genet. 38, 1005 – 14.
214 Schmid, M., Arib, G., Laemmli, C., Nishikawa, J., Durussel, T. and Laemmli, U.K. (2006) Nup-PI: the nucleopore-promoter interaction of genes in yeast. Mol. Cell 21, 379 – 91.
215 Casolari, J.M., Brown, C.R., Drubin, D.A., Rando, O.J. and Silver, P.A. (2005) Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. Genes Dev. 19, 1188 – 98.
216 Casolari, J.M., Brown, C.R., Komili, S., West, J., Hieronymus, H. and Silver, P.A. (2004) Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell 117, 427 – 39.
217 Lucchesi, J.C., Kelly, W.G. and Panning, B. (2005) Chromatin remodeling in dosage compensation. Annu. Rev. Genet. 39, 615 – 51.
218 Rea, S. and Akhtar, A. (2006) MSL proteins and the regulation of gene expression. Curr. Top. Microbiol. Immunol. 310, 117 – 40.
219 Mendjan, S., Taipale, M., Kind, J., Holz, H., Gebhardt, P., Schelder, M., Vermeulen, M., Buscaino, A., Duncan, K., Mueller, J., Wilm, M., Stunnenberg, H.G., Saumweber, H. and Akhtar, A. (2006) Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. Mol. Cell 21, 811 – 23.
220 Taddei, A., Van Houwe, G., Hediger, F., Kalck, V., Cubizolles, F., Schober, H. and Gasser, S.M. (2006) Nuclear pore association confers optimal expression levels for an inducible yeast gene. Nature 441, 774 – 8.
221 Galy, V., Olivo-Marin, J.C., Scherthan, H., Doye, V., Rascalou, N. and Nehrbass, U. (2000) Nuclear pore complexes in the organization of silent telomeric chromatin. Nature 403, 108 – 12.
222 Therizols, P., Fairhead, C., Cabal, G.G., Genovesio, A., Olivo-Marin, J.C., Dujon, B. and Fabre, E. (2006) Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol. 172, 189 – 99.
223 Feuerbach, F., Galy, V., Trelles-Sticken, E., Fromont-Racine, M., Jacquier, A., Gilson, E., Olivo-Marin, J.C., Scherthan, H. and Nehrbass, U. (2002) Nuclear architecture and spatial positioning help establish transcriptional states of telomeres in yeast. Nat. Cell Biol. 4, 214 – 21.
224 Gotta, M., Laroche, T., Formenton, A., Maillet, L., Scherthan, H. and Gasser, S.M. (1996) The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J. Cell Biol. 134, 1349 – 63.
225 Gottschling, D.E., Aparicio, O.M., Billington, B.L. and Zakian, V.A. (1990) Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription. Cell 63, 751 – 62.
226 Laroche, T., Martin, S.G., Gotta, M., Gorham, H.C., Pryde, F.E., Louis, E.J. and Gasser, S.M. (1998) Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. Curr. Biol. 8, 653 – 6.
227 Bystricky, K., Laroche, T., van Houwe, G., Blaszczyk, M. and Gasser, S.M. (2005) Chromosome looping in yeast: telomere pairing and coordinated movement reflect anchoring efficiency and territorial organization. J. Cell Biol. 168, 375 – 87.
228 Capelson, M. and Corces, V.G. (2005) The ubiquitin ligase dTopors directs the nuclear organization of a chromatin insulator. Mol. Cell 20, 105 – 16.
229 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 – 8.
230 Lei, E.P. and Corces, V.G. (2006) RNA interference machinery influences the nuclear organization of a chromatin insulator. Nat. Genet. 38, 936 – 41.
231 Gurdon, J.B. (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. J. Embryol. Exp. Morphol. 10, 622 – 40.
232 Meshorer, E., Yellajoshula, D., George, E., Scambler, P.J., Brown, D.T. and Misteli, T. (2006) Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev. Cell 10, 105 – 16.
233 Dean, W., Santos, F., Stojkovic, M., Zakhartchenko, V., Walter, J., Wolf, E. and Reik, W. (2001) Conservation of methylation reprogramming in mammalian development: aberrant reprogramming in cloned embryos. Proc. Natl. Acad. Sci. USA 98, 13734 – 8.
234 Hochedlinger, K. and Jaenisch, R. (2006) Nuclear reprogramming and pluripotency. Nature 441, 1061 – 7.
235 Ogonuki, N., Inoue, K., Yamamoto, Y., Noguchi, Y., Tanemura, K., Suzuki, O., Nakayama, H., Doi, K., Ohtomo, Y., Satoh, M., Nishida, A. and Ogura, A. (2002) Early death of mice cloned from somatic cells. Nat. Genet. 30, 253 – 4.
236 Tamashiro, K.L., Wakayama, T., Akutsu, H., Yamazaki, Y., Lachey, J.L., Wortman, M.D., Seeley, R.J., D'Alessio, D.A., Woods, S.C., Yanagimachi, R. and Sakai, R.R. (2002) Cloned mice have an obese phenotype not transmitted to their offspring. Nat Med 8, 262 – 7.
237 Brambrink, T., Hochedlinger, K., Bell, G. and Jaenisch, R. (2006) ES cells derived from cloned and fertilized blastocysts are transcriptionally and functionally indistinguishable. Proc. Natl. Acad. Sci. USA 103, 933 – 8.
238 Carninci, P., Kasukawa, T., Katayama, S., Gough, J., Frith, M.C., Maeda, N., Oyama, R., Ravasi, T., Lenhard, B., Wells, C., Kodzius, R., Shimokawa, K., Bajic, V.B., Brenner, S.E., Batalov, S., Forrest, A.R., Zavolan, M., Davis, M.J., Wilming, L.G., Aidinis, V., Allen, J.E., Ambesi-Impiombato, A., Apweiler, R., Aturaliya, R.N., Bailey, T.L., Bansal, M., Baxter, L., Beisel, K.W., Bersano, T., Bono, H., Chalk, A.M., Chiu, K.P., Choudhary, V., Christoffels, A., Clutterbuck, D.R., Crowe, M.L., Dalla, E., Dalrymple, B.P., de Bono, B., Della Gatta, G., di Bernardo, D., Down, T., Engstrom, P., Fagiolini, M., Faulkner, G., Fletcher, C.F., Fukushima, T., Furuno, M., Futaki, S., Gariboldi, M., Georgii-Hemming, P., Gingeras, T.R., Gojobori, T., Green, R.E., Gustincich, S., Harbers, M., Hayashi, Y., Hensch, T.K., Hirokawa, N., Hill, D., Huminiecki, L., Iacono, M., Ikeo, K., Iwama, A., Ishikawa, T., Jakt, M., Kanapin, A., Katoh, M., Kawasawa, Y., Kelso, J., Kitamura, H., Kitano, H., Kollias, G., Krishnan, S.P., Kruger, A., Kummerfeld, S.K., Kurochkin, I.V., Lareau, L.F., Lazarevic, D., Lipovich, L., Liu, J., Liuni, S., McWilliam, S., Madan Babu, M., Madera, M., Marchionni, L., Matsuda, H., Matsuzawa, S., Miki, H., Mignone, F., Miyake, S., Morris, K., Mottagui-Tabar, S., Mulder, N., Nakano, N., Nakauchi, H., Ng, P., Nilsson, R., Nishiguchi, S., Nishikawa, S., Nori, F., Ohara, O., Okazaki, Y., Orlando, V., Pang, K.C., Pavan, W.J., Pavesi, G., Pesole, G., Petrovsky, N., Piazza, S., Reed, J., Reid, J.F., Ring, B.Z., Ringwald, M., Rost, B., Ruan, Y., Salzberg, S.L., Sandelin, A., Schneider, C., Schonbach, C., Sekiguchi, K., Semple, C.A., Seno, S., Sessa, L., Sheng, Y., Shibata, Y., Shimada, H., Shimada, K., Silva, D., Sinclair, B., Sperling, S., Stupka, E., Sugiura, K., Sultana, R., Takenaka, Y., Taki, K., Tammoja, K., Tan, S.L., Tang, S., Taylor, M.S., Tegner, J., Teichmann, S.A., Ueda, H.R., van Nimwegen, E., Verardo, R., Wei, C.L., Yagi, K., Yamanishi, H., Zabarovsky, E., Zhu, S., Zimmer, A., Hide, W., Bult, C., Grimmond, S.M., Teasdale, R.D., Liu, E.T., Brusic, V., Quackenbush, J., Wahlestedt, C., Mattick, J.S., Hume, D.A., Kai, C., Sasaki, D., Tomaru, Y., Fukuda, S., Kanamori-Katayama, M., Suzuki, M., Aoki, J., Arakawa, T., Iida, J., Imamura, K., Itoh, M., Kato, T., Kawaji, H., Kawagashira, N., Kawashima, T., Kojima, M., Kondo, S., Konno, H., Nakano, K., Ninomiya, N., Nishio, T., Okada, M., Plessy, C., Shibata, K., Shiraki, T., Suzuki, S., Tagami, M., Waki, K., Watahiki, A., Okamura-Oho, Y., Suzuki, H., Kawai, J. and Hayashizaki, Y. (2005) The transcriptional landscape of the mammalian genome. Science 309, 1559 – 63.
239 Carninci, P., Sandelin, A., Lenhard, B., Katayama, S.,

Shimokawa, K., Ponjavic, J., Semple, C.A., Taylor, M.S., Engstrom, P.G., Frith, M.C., Forrest, A.R., Alkema, W.B., Tan, S.L., Plessy, C., Kodzius, R., Ravasi, T., Kasukawa, T., Fukuda, S., Kanamori-Katayama, M., Kitazume, Y., Kawaji, H., Kai, C., Nakamura, M., Konno, H., Nakano, K., Mottagui-Tabar, S., Arner, P., Chesi, A., Gustincich, S., Persichetti, F., Suzuki, H., Grimmond, S.M., Wells, C.A., Orlando, V., Wahlestedt, C., Liu, E.T., Harbers, M., Kawai, J., Bajic, V.B., Hume, D.A. and Hayashizaki, Y. (2006) Genome-wide analysis of mammalian promoter architecture and evolution. Nat. Genet. 38, 626 – 35.

240 Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663 – 76.

241 Eggan, K., Baldwin, K., Tackett, M., Osborne, J., Gogos, J., Chess, A., Axel, R. and Jaenisch, R. (2004) Mice cloned from olfactory sensory neurons. Nature 428, 44 – 9.

242 Hochedlinger, K., Blelloch, R., Brennan, C., Yamada, Y., Kim, M., Chin, L. and Jaenisch, R. (2004) Reprogramming of a melanoma genome by nuclear transplantation. Genes Dev. 18, 1875 – 85.

243 Baxter, J., Sauer, S., Peters, A., John, R., Williams, R., Caparros, M.L., Arney, K., Otte, A., Jenuwein, T., Merkenschlager, M. and Fisher, A.G. (2004) Histone hypomethylation is an indicator of epigenetic plasticity in quiescent lymphocytes. EMBO J. 23, 4462 – 72.

244 Blelloch, R., Wang, Z., Meissner, A., Pollard, S., Smith, A. and Jaenisch, R. (2006) Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus. Stem Cells 24, 2007 – 13.

245 Molofsky, A. V., Pardal, R., Morrison, S. J. (2004) Diverse mechanisms regulate stem cell self-renewal. Curr. Opin. Cell Biol. 16, 700 – 7.

246 Mishra, L., Derynck, R., Mishra, B. (2005) Transforming growth factor-beta signaling in stem cells and cancer. Science 310, 68 – 71.

247 Maurange, C., Lee, N., Paro, R. (2006) Signaling meets chromatin during tissue regeneration in Drosophila. Curr. Opin. Genet. Dev. 16, 485 – 9.

248 Lee, N., Maurange, C., Ringrose, L. and Paro, R. (2005) Suppression of Polycomb group proteins by JNK signalling induces transdetermination in *Drosophila* imaginal discs. Nature 438, 234 – 7.

249 Sustar, A. and Schubiger, G. (2005) A transient cell cycle shift in *Drosophila* imaginal disc cells precedes multipotency. Cell 120, 383 – 93.

250 Lund, A.H. and van Lohuizen, M. (2004) Epigenetics and cancer. Genes Dev. 18, 2315 – 35.

251 Martinez, A.M. and Cavalli, G. (2006) The role of polycomb group proteins in cell cycle regulation during development. Cell Cycle 5, 1189 – 97.

252 Martinez, A.M., Colomb, S., Dejardin, J., Bantignies, F. and Cavalli, G. (2006) Polycomb group-dependent cyclin A repression in *Drosophila*. Genes Dev. 20, 501 – 13.

253 Lemaitre, J.M., Danis, E., Pasero, P., Vassetzky, Y. and Mechali, M. (2005) Mitotic remodeling of the replicon and chromosome structure. Cell 123, 787 – 801.

254 Buongiorno-Nardelli, M., Micheli, G., Carri, M.T. and Marilley, M. (1982) A relationship between replicon size and supercoiled loop domains in the eukaryotic genome. Nature 298, 100 – 2.