REVIEW

Epigenetics and cancer

Anders H. Lund[1,2,3] and Maarten van Lohuizen[1,4]

[1]Division of Molecular Genetics, The Netherlands Cancer Institute, 1066CX Amsterdam, The Netherlands; [2]Biotech Research & Innovation Centre, Symbion Science Park, 2100 Copenhagen, Denmark

Epigenetic mechanisms act to change the accessibility of chromatin to transcriptional regulation locally and globally via modifications of the DNA and by modification or rearrangement of nucleosomes. Epigenetic gene regulation collaborates with genetic alterations in cancer development. This is evident from every aspect of tumor biology including cell growth and differentiation, cell cycle control, DNA repair, angiogenesis, migration, and evasion of host immunosurveillance. In contrast to genetic cancer causes, the possibility of reversing epigenetic codes may provide new targets for therapeutic intervention.

Epigenetic programming is crucial in mammalian development, and stable inheritance of epigenetic settings is essential for the maintenance of tissue- and cell-type-specific functions (Li 2002). With the exception of controlled genomic rearrangements, such as those of the immunoglobulin and T-cell receptor genes in B and T cells, all other differentiation processes are initiated or maintained through epigenetic processes. Not surprisingly therefore, epigenetic gene regulation is characterized overall by a high degree of integrity and stability. Evidence is accumulating that suggests that the intrinsic stability is caused by multiple interlocking feedback mechanisms between functionally unrelated epigenetic layers, such as DNA methyltransferases (DNMTs) and histone modifying enzymes, resulting in the stable commitment of a locus to a particular activity state. In somatic cells, the transcriptional status of most genes is epigenetically fixed. However, other genes, such as cell cycle checkpoint genes and genes directly affected by exogenous stimuli such as growth factors or cell–cell contact, likely reside in a balanced state sensitive to dynamic adjustments in histone modifications, thereby allowing for rapid responses to specific stimuli. Perturbation of epigenetic balances may lead to alterations in gene expression, ultimately resulting in cellular transformation and malignant outgrowth; the involvement of deregulated epigenetic mechanisms in cancer development has received increased attention in recent years.

Per definition, epigenetic regulators alter the activities and abilities of a cell without directly affecting and mutating the sequence of the DNA. In this review, we deal with epigenetic gene regulation as imposed by DNA methylation, covalent modifications of the canonical core histones, deposition of variant histone proteins, local nucleosome remodeling, and long-range epigenetic regulators. Understanding the molecular details behind "epigenetic cancer diseases" holds potentially important prospects for medical treatment, as it allows for novel strategies for drug development. A number of recent reviews have provided details on epigenetic mechanisms and their involvement in cancer, and we refer to these for more in-depth information on individual epigenetic mechanisms (Bird 2002; Jones and Baylin 2002; Jaenisch and Bird 2003; Feinberg and Tycko 2004; Hake et al. 2004).

Epigenetic gene regulation

Epigenetic gene regulation has the nucleosome on center stage. The nucleosome is made up of approximately two turns of DNA wrapped around a histone octamer built from two subunits of each histone, H2A, H2B, H3, and H4, respectively, In between core nucleosomes, the linker histone H1 attaches and facilitates further compaction (for a recent review, see Khorasanizadeh 2004). Aside from the core histones, a variety of variant histone proteins exist and can be inserted into the nucleosome, possibly serving as landmarks for specific cellular functions (discussed following). The N-terminal tails of the histone proteins are protruding out from the nucleosomal core particles, and these tails serve as regulatory registers onto which epigenetic signals can be written. Covalent modification of histones includes acetylation of lysines, methylation of lysines and arginines, phosphorylations of serines and threonines, ADP-ribosylation of glutamic acids, and ubiquitination and sumolyation of lysine residues (Fig. 1). The pattern of histone modifications signifies the status of the chromatin locally and has been coined the histone code (Strahl and Allis 2000). A second group of proteins, containing bromodomain and chromodomain modules, use the epigenetic marks on the histone tails as recognition landmarks to bind the chromatin and initiate downstream biological processes such as chromatin compaction, transcriptional regulation, or DNA repair (Fig. 2). Al-
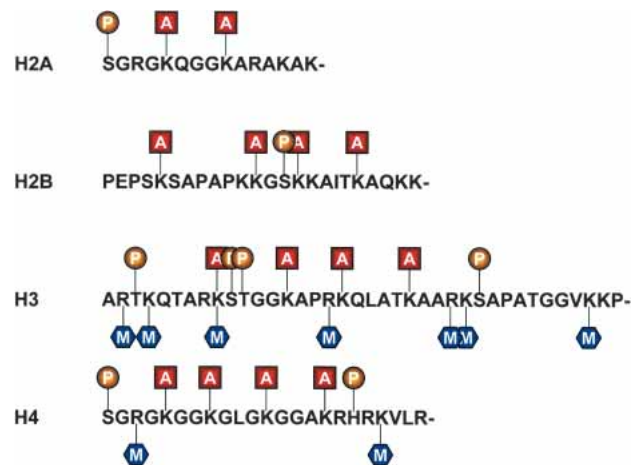
Figure 1. Covalent modifications of the N-terminal tail of the canonical core histones. Phosphorylations are shown as yellow circles, acetylations as red squares, and methylations as blue hexagons.

though histone acetylation in general correlates with transcriptional activity, histone methylations can serve as anchorage points for both activator and repressor complexes and are thereby involved in conferring an epigenetic inheritance to the cell, carrying information related to differentiation commitment and function. Although most histone modifications are reversible, no histone demethylase has been identified as yet, suggesting histone methylation to be involved in processes of long-term regulation.

Histone imprints impinge on each other. This is exemplified in gene activation where phosphorylation of histone H3 Ser 10 facilitates acetylation of Lys 14 and methylation of Lys 4, resulting in an open chromatin conformation (Cheung et al. 2000; Lo et al. 2000). Ser 10 phosphorylation also facilitates acetylation of Lys 9, thereby preventing the setting of repressive Lys 9 methylation marks (Rea et al. 2000). Hence, some histone modifications may act as molecular switches, enabling or blocking the setting of other covalent marks (Fischle et al. 2003). Also DNA methylation affects histone modifications and vice versa, to make up a highly intricate epigenetic control mechanism, the impact of which we have only just begun to comprehend.

In the following sections, we describe emerging themes involving variant histone proteins and RNA-guided epigenetic mechanisms, and we review some of the known connections between epigenetic gene regulators and cancer.

Histone modification: setting the mark

Histone proteins can be mono-, di-, or trimethylated at the ε amino group of lysine residues and either mono- or dimethylated at arginine residues. In combination with other covalent modifications such as acetylation, phosphorylation, and ubiquitination, methylations of histone proteins are thought to represent an epigenetic code by the creation of binding interfaces for proteins involved in chromatin regulation (Strahl and Allis 2000; Jenuwein and Allis 2001; Lachner et al. 2003). Several SET (SuVar39, Enhancer of Zeste, and Trithorax) domain proteins have been demonstrated to be methyltransferases capable of covalently altering the lysine residues of histone proteins. Many SET domain proteins have been tightly linked to cancer development (Huang 2002; Schneider et al. 2002) and in the following section, the most prominent cases are briefly reviewed.

Setting Polycomb imprints

Polycomb-group (PcG) proteins form transcriptional repressor modules that functionally can be divided into at least two distinct complexes: the initiation complex, Polycomb repression complex 2 (PRC2), the core of which in humans consists of EZH2, EED, and SUZ12; and the maintenance complex, PRC1, with the core proteins RNF2, HPC, EDR, and BMI1. Both PRC1 and PRC2 complex members have been linked to cell cycle control and cancer. Together with the Trithorax group (TrxG) of proteins, which form positively acting transcriptional regulators (see following), the PcG complexes are thought to constitute a cellular memory system responsible for maintaining the epigenetic status of target genes throughout the lifetime of the organism (Jacobs and van Lohuizen 1999; Orlando 2003; Lund and van Lohuizen 2004). Members of both PcG and TrxG protein complexes harbor SET domains. Recent data have demonstrated that the SET domain present in EZH2 is responsible for methylation of Lys 9 and, more prominently, Lys 27 of histone H3 (Cao et al. 2002; Czermin et al. 2002; Kuzmichev et al. 2002; Muller et al. 2002) and Lys 26 on histone H1 (Kuzmichev et al. 2004). Interestingly, emerging data suggest that the PRC2 complex composition may influence the type of methylation imprint imposed by EZH2, thereby adding yet another level of regulation (Kuzmichev et al. 2004).

EZH2 is overexpressed in many cancers (Varambally et al. 2002; Bracken et al. 2003; Kleer et al. 2003; Raaphorst et al. 2003) and the expression level of EZH2 correlates with a poorer prognosis in both prostate (Varambally et al. 2002) and breast cancer (Kleer et al. 2003). Surprisingly, although PRC2 complex members are directly controlled by the RB tumor-suppressor pathway, their expression levels do not appear to fluctuate in a cell cycle-dependent manner (Weinmann et al. 2001; Bracken et al. 2003). RNAi-mediated knockdown of EZH2 or EED in human primary or transformed cells results in a block of proliferation (Varambally et al. 2002; Bracken et al. 2003), and the observed overexpression of EZH2 in cancers is likely causally related to tumorigenesis and not a mere consequence of deregulated RB–E2F functioning, as the EZH2 locus is amplified in several cancers (Bracken et al. 2003). This, however, remains to be confirmed in mouse models. The oncogenic mechanism underlying EZH2 overexpression is unknown. It has been speculated that EZH2 overexpression may exert dominant negative
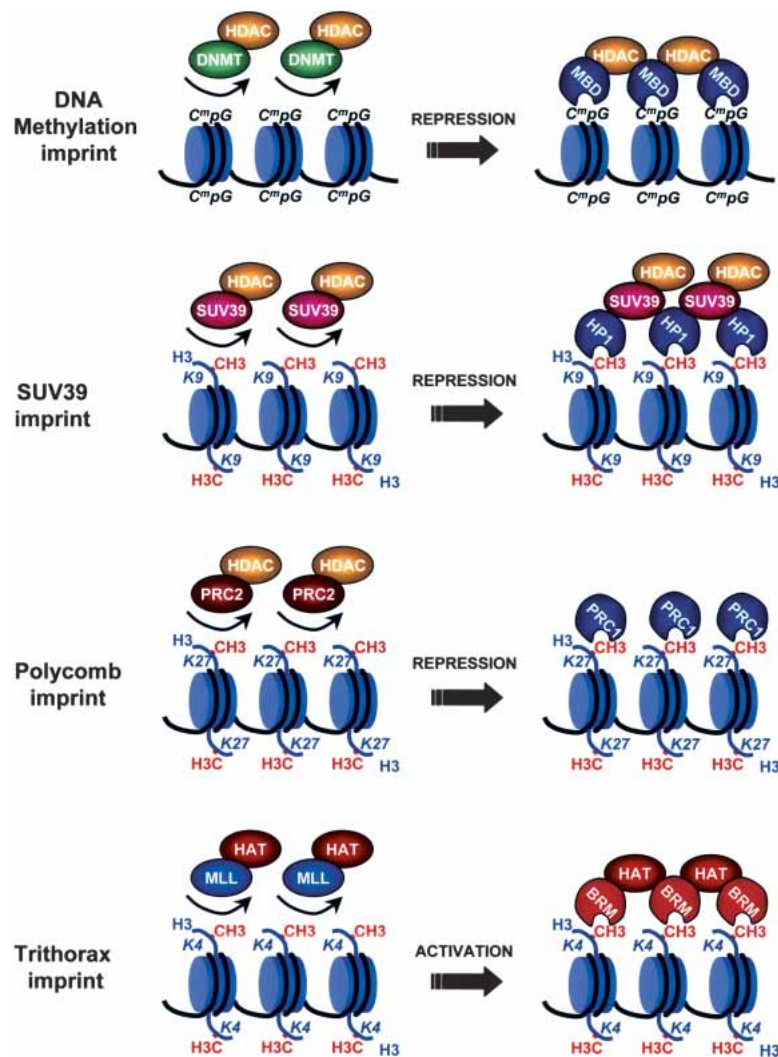
Figure 2. Epigenetic gene regulation. Nucleosomal arrays depicting proteins known to be involved in epigenetic gene regulation. On the *left*-hand side are proteins involved in setting the histone code and on the *right*-hand side are proteins capable of reading histone imprints. See text for details.

effects on PRC2 complex functions (Kuzmichev et al. 2004); however, experimental overexpression of EZH2 results in target gene suppression only (Varambally et al. 2002), and hence does not sustain a notion of dominant negative effects. The PRC2 complex is also required for X-chromosome inactivation (Plath et al. 2003; Silva et al. 2003) and for correct imprinting of autosomal loci (Wang et al. 2001b). These functions point to long-range mechanisms of transcriptional repression and suggest that PRC2 activity may have global consequences to the cell, perhaps via more generally skewing the balance between specific histone methylation imprints.

Setting Trithorax imprints

The human homologs of the *Drosophila* Trithorax genes are well known to cancer research. The mixed lineage leukemia gene (MLL1, also known as HRX or ALL1) is involved in 11q23 translocations in acute leukemias (Djabali et al. 1992; Gu et al. 1992; Tkachuk et al. 1992), and the other four human MLL homologs all reside in cancer-related loci.

Trimethylation of Lys 4 on histone H3 correlates with transcriptional activity (Santos-Rosa et al. 2002), and MLL1 was recently shown to be a histone H3 Lys 4-specific methyltransferase (Milne et al. 2002; Nakamura et al. 2002). MLL1 has been found to associate with other epigenetic regulators, such as the HAT CBP (Ernst et al. 2001) and INI1 (Rozenblatt-Rosen et al. 1998), a subunit of the SWI/SNF nucleosome remodeling complex, and one study found MLL1 to reside in a large protein assembly containing subunits capable of regulating transcription preinitiation, nucleosome remodeling, histone acetylation, and histone methylation (Nakamura et al. 2002). A similarly orchestrated epigenetic regulation was demonstrated for the *Drosophila* Trithorax-like protein Ash1, a histone methyltransferase (HMT) capable of methylating Lys 4 and Lys 9 of histone H3 and Lys 20 on histone H4 (Beisel et al. 2002). The Ash1-specific methylation imprint was found to displace HP1 and PcG proteins, which are normally bound to repressed genes, and instead facilitates the binding of the Brahma chromatin remodeling complex (Beisel et al. 2002). Interestingly, recent data from *Drosophila* studies

indicate that TrxG proteins such as Trx and Ash1, rather than being general transcriptional coactivators, specifically function to prevent inappropriate silencing mediated by the PcG of transcriptional repressors (Klymenko and Muller 2004).

More than 40 specific translocations involving MLL1 have been described in human cancers to date (Ayton and Cleary 2001). Intriguingly, all fusion proteins retain the N-terminal part of MLL1, whereas the C-terminal part encoding the SET domain is lost. The oncogenic capacity of several MLL fusion proteins has been verified in transgenic mice (Corral et al. 1996; Dobson et al. 1999). Mll heterozygous mice do not appear tumor prone, making haploinsufficiency a less likely cause of leukemogenesis (Corral et al. 1996). Rather, the fusion proteins appear to exert dominant-negative functions over wild-type MLL (Arakawa et al. 1998; Ayton and Cleary 2001). It remains enigmatic how MLL fusion proteins retain transcription activation capacity while lacking a functional SET domain and how structurally divergent fusion partners of MLL1 can result in relatively similar consequences in terms of cellular transformation. Recent data have demonstrated that dimerization of the MLL fusion partners may be an important virtue in some translocations (So et al. 2003). This could explain the diversity in MLL fusion partners, but leaves open the question of which targets are selectively being (de)regulated by the MLL fusion proteins as compared with the wild-type MLL. Intriguingly, the known MLL target genes *HoxA7* and *HoxA9* are both required for leukemia induction by an MLL fusion oncogene (Ayton and Cleary 2003). In other MLL translocation products, the fusion partner moiety is likely capable of sequestering coactivators or nucleosome remodeling factors contributing to oncogenesis (discussed in So and Cleary 2004).

SUV39H1 is the mammalian homolog of the *Drosophila* position effect variegation modifier Su(var)3-9 and was the first SET domain protein to be recognized as a histone methyl transferase (Rea et al. 2000). The fundamental importance of this finding is reflected in the current intense research interest in HMTs. SUV39H1 is involved in stabilizing heterochromatic regions of the genome via trimethylation of histone H3 Lys 9 (Rea et al. 2000; Peters et al. 2001). This methyl imprint creates an anchor point for chromodomain proteins of the HP1 family (Bannister et al. 2001; Lachner et al. 2001). This ancient repressive system is self-sustaining in a cyclic manner, as HP1 associates directly with SUV39H1. Mice deficient for the two Suv39 homologs display chromosomal instability and are prone to develop B-cell lymphomas resembling human non-Hodgkin lymphomas, albeit with long latency (Peters et al. 2001). Whereas Suv39h deficiency in the mouse predominantly was found to affect heterochromatin stability, the Suv39h methyltransferases associate with numerous proteins involved in cell cycle regulation including pRB (Nielsen et al. 2001; Vandel et al. 2001), PcG-proteins (Sewalt et al. 2002), and SMADs (Frontelo et al. 2004). Tumor-derived mutants of pRB have been identified that do not bind

SUV39H proteins, signifying the importance of the association (Nielsen et al. 2001). Recently, Suv39h1/2 was also shown to be important for regulating the length of mammalian telomeres (Garcia-Cao et al. 2004). The reduced histone H3 methylation resulting from loss of Suv39h caused a decrease in HP1 association to the telomeres and abnormal telomere elongation (Garcia-Cao et al. 2004). In embryonic stem (ES) cells lacking both Suv39 homologs the pericentromeric heterochromatin is devoid of the characteristic trimethylation of histone H3, Lys 9. Instead, an alternative methylation imprint consisting of monomethylated Lys 9 and trimethylated Lys 27 has accumulated (Peters et al. 2003). This suggests a functional redundancy between different epigenetic gene repression mechanisms, which may serve to explain why loss of Suv39h-mediated repression does not have an even stronger phenotype.

RIZ1, originally isolated from its interaction with the RB tumor suppressor protein (Buyse et al. 1995), was the first SET domain protein demonstrated to be a tumor suppressor (Steele-Perkins et al. 2001). The *RIZ1* SET domain was recently shown to be a histone H3 Lys 9-specific methyltransferase (Kim et al. 2003). The *RIZ* gene is situated on chromosome 1p36 in one of the most frequently deleted regions in human cancers (Buyse et al. 1996; Huang 2002), and *RIZ* has been found to be inactivated by mutations or DNA methylation in a wide variety of human tumors including breast cancer, liver cancer, colorectal cancer, lung cancer, lymphomas, and melanomas (Chadwick et al. 2000; Du et al. 2001; Poetsch et al. 2002; Sasaki et al. 2002; Oshimo et al. 2004). The *RIZ* gene produces two proteins, RIZ1 and RIZ2, because of the use of alternative promoters. Interestingly, only RIZ1 harbors a SET domain and appears to be the main target of inactivation in tumors (Huang 2002), and mice lacking Riz1 function only are prone to tumor formation (Steele-Perkins et al. 2001). In accordance with a tumor-suppressive function of RIZ1, ectopic expression in cancer cell lines results in cell cycle arrest and/or apoptosis (He et al. 1998; Jiang et al. 1999).

SMYD3 was recently identified as a SET and MYND domain-containing protein overexpressed in colorectal carcinomas and hepatocellular carcinomas (Hamamoto et al. 2004). SMYD3 was found to interact with the RNA polymerase II complex and activate target genes via methylation of histone H3 Lys 4. Interestingly, the MYND domain appears to confer sequence-specific binding to target genes. Whereas the oncogenic capacity of SMYD3 is yet to be confirmed in an in vivo model, overexpression of SMYD3 results in colony formation of NIH 3T3 cells. Conversely, siRNA-mediated knockdown of SMYD3 in cell lines derived from hepatomas and colorectal cancers was shown to have growth-inhibitory effects (Hamamoto et al. 2004).

MDS-EVI1 was originally annotated as two independent genes. Evi1 was characterized in the mouse as a cancer-related retroviral integration site in myeloid leukemia (Morishita et al. 1988), and EVI1 was subsequently identified as a translocation breakpoint in hu-

mans (Morishita et al. 1992). The 5′ MDS part of MDS-EVI1 contains a SET domain, and both retroviral integrations in the mouse and translocations in humans result in the selective overexpression of the EVI1 variant lacking a SET domain. EVI1 acts dominantly over its presumed antagonist MDS-EVI1 (Soderholm et al. 1997; Sitailo et al. 1999), and high EVI1 expression correlates with poor prognosis in patients with acute myeloid leukemia (Barjesteh van Waalwijk van Doorn-Khosrovani et al. 2003).

An emerging central theme is that several SET domain proteins can be found in a cancer-promoting and a cancer-preventing isoform. This is true for the RIZ and MDS-EVI1 genes, both of which contain two promoters dictating separate protein isoforms, one form holding and one form lacking the SET domain. The same picture emerges from the translocation fusion proteins involving MLL, all of which lack the SET protein domain. In addition, cancer-specific translocations of the SET domain protein MMSET/NSD2 have been characterized, in which the SET domain is selectively lost (Bergsagel and Kuehl 2001). Together, these findings have led to the ying-yang theory (Huang 2002), proposing that the natural homeostasis existing between protein isoforms with and without SET domain is perturbed in cancers, resulting in selective expression of the oncogenic over the tumor-suppressive isoform.

Arginine methyltransferases

Although no arginine-specific methyltransferases have been implicated in cancer development yet, several of these enzymes are involved in gene regulatory complexes important for cell cycle regulation. PRMT4/CARM1 positively regulates transcription of, among others, estrogen receptor-responsive genes via binding to the histone acetyl transferase CPB/p300 and methylation of Arg 17 of histone H3 (Xu et al. 2001; Daujat et al. 2002). The PRMT5 arginine methyltransferase can methylate both histone H3 and H4 in vitro and interacts directly with components of the SWI/SNF complex (Pal et al. 2003). PRMT5 was furthermore found to associate with and negatively regulate the cyclin E promoter (Fabbrizio et al. 2002). It is unknown whether methylated arginines form recognition sites for chromatin-associated proteins. Alternatively, arginine methylations may regulate other histone codes. To this end, methylation of Arg 3 of histone H4 by PRMT1 has been found to facilitate H4 acetylation and enhance transcriptional activity (Wang et al. 2001a). It is conceivable, therefore, that arginine methyltransferases also will display links to cancer once we learn more about these enzymes.

Histone acetylation

Acetylation of histone proteins correlates with transcriptional activation and a dynamic equilibrium of histone acetylation is governed by the opposing actions of HATs and histone deacetylases (HDACs) (for recent reviews, see Marks et al. 2001; Yang 2004). Aside from histones, many transcriptional regulators, chromatin modifiers, and intracellular signal transducers are posttranslationally modified by acetylation. Both HATs and HDACs have been found mutated or deregulated in various cancers.

The two closely related HATs, p300 and CBP, act as transcriptional cofactors for a range of cellular oncoproteins, such as MYB, JUN, FOS, RUNX, BRCA1, p53, and pRB, as well as for the viral oncoproteins E1A, E6, and SV40 large T (for review, see Caron et al. 2003; Iyer et al. 2004). CBP and p300 are functional tumor suppressors as demonstrated by several lines of evidence. Both genes reside in regions frequently lost in tumors, and cancer-specific mutations abolishing the enzymatic activity of p300 have been identified (Muraoka et al. 1996; Gayther et al. 2000; Ozdag et al. 2002). CBP and p300 are found disrupted by translocations in leukemia with translocation partners including MLL, MOZ, and MORF (Borrow et al. 1996; Sobulo et al. 1997; Panagopoulos et al. 2001). Germ-line mutations in *CBP* causes the developmental disorder Rubenstein-Taybi syndrome, and these patients suffer an increased cancer risk (Petrij et al. 1995; Murata et al. 2001). Finally, genetic ablation studies of *Cbp* and *p300* in mouse models have confirmed that both proteins function as tumor suppressors (Yao et al. 1998; Kung et al. 2000; Kang-Decker et al. 2004).

HDACs have, not unlike DNA methylation, dualistic and opposite functions in cancer development. On the one hand, HDACs play prominent roles in the transcriptional inactivation of tumor-suppressor genes. This is evident from studies using pharmacological inhibitors of HDAC activity in cancer therapies (discussed following). On the other hand is the reliance of important tumor-suppressor mechanisms on HDAC function, as exemplified by the dependency of RB on HDAC1 for transcriptional repression of E2F target genes (Luo et al. 1998; Magnaghi-Jaulin et al. 1998; Robertson et al. 2000). *Hdac1*-deficient mice are not viable and ES cells with homozygous *Hdac1* deletion display proliferation defects correlating with increased levels of the cyclin-dependent kinase inhibitors p21 and p27 (Lagger et al. 2002), demonstrating the involvement of HDAC1 in cell cycle regulation. Recently, Hdac2 was genetically linked to the Wnt pathway, as Hdac2 is overexpressed in tumors and tissues from mice lacking the adenomatosis polyposis coli (APC) tumor suppressor (Zhu et al. 2004a). Likewise, RNAi-mediated knockdown of HDAC2 in colonic cancer cells resulted in cell death, indicating a role for HDAC2 in protecting cancer cells against apoptosis (Zhu et al. 2004a).

Importantly, HDACs are associated with a number of other epigenetic repression mechanisms, including histone methylation (Ogawa et al. 2002; Vaute et al. 2002), PcG-mediated repression (van der Vlag and Otte 1999), and DNA methylation (Fuks et al. 2000; Rountree et al. 2000). Importantly, HDAC activity is often crucial to prepare the histone template for methyltransferases by removing acetyl groups obstructing methylation. HDACs are, moreover, often found as "partners in

crime" when captured by oncoproteins such as PML–RARα or AML–ETO to induce aberrant gene silencing (see following).

DNA methylation

DNA methylation remains the best-studied epigenetic mechanism. Methylation at the C-5 position of cytosine residues present in CpG dinucleotides by DNMTs has evolved relatively late in evolution and is generally considered to facilitate static long-term gene silencing and to confer genome stability through repression of transposons and repetitive DNA elements (Yoder et al. 1997). CpG dinucleotides are statistically underrepresented in the genome, likely because methylation of cytosine makes them more susceptible to deamination, but are locally enriched in shorter stretches of DNA (0.5 to several kilobases) known as CpG islands (Bird 2002). CpG islands are most often associated with promoter regions and an estimated 60% of human genes contain a CpG island (Antequera and Bird 1993). Patterns of DNA methylation are laid out during early embryogenesis via the interplay of at least three active DNA methyltransferases, DNMT1, DNMT3A, and DNMT3B, and targeted deletions of these genes in the mouse have demonstrated that the enzymes are essential for normal development (Li et al. 1992; Okano et al. 1999). DNMT1 associates with the DNA replication fork and functions as a hemi-methylase responsible for maintaining correct methylation patterns on DNA replication. DNMT3A and DNMT3B are instead de novo methyltransferases important for establishing methylation patterns during embryogenesis, and they associate with the replication fork in late S-phase during the replication of constitutive heterochromatin (Bachman et al. 2001).

DNA methylation is tightly connected to cancer development and can act as a double-edged sword. On the one hand, oncogenesis is promoted by local hypermethylation of tumor-suppressor genes, whereas global genomic hypomethylation, on the other hand, affects oncogene expression and genomic stability (Jones and Baylin 2002). All three methyltransferase enzymes have been found to be overexpressed in human tumors, although to moderate levels only (De Marzo et al. 1999; Robertson et al. 1999; Girault et al. 2003; Nagai et al. 2003). Dnmt1 was shown to be necessary and sufficient for Fos-induced transformation of mouse fibroblast cells (Bakin and Curran 1999), and DNMT1 knockdown experiments in human cancer cell lines have demonstrated an essential role for DNMT1 in maintaining aberrant repression of tumor-suppressor loci (Robert et al. 2003). Likewise, cell culture studies have demonstrated an important role for DNMT3A in cancer cell survival (Beaulieu et al. 2002). Germ-line mutations in *DNMT3B* underlies ICF syndrome, a rare disorder characterized by immunodeficiency, centromeric instability, and facial abnormalities (Hansen et al. 1999; Xu et al. 1999). Interestingly, mouse embryo fibroblasts deficient for Dnmt3b

are resistant to transformation by SV40 largeT and activated Ras oncogenes (Soejima et al. 2003), demonstrating that epigenetic and genetic mechanisms likely act in concert during cellular transformation.

Ectopic cytosine hypermethylation is generally associated with transcriptional repression and, ultimately, tumor formation. Collaboration between genetic and epigenetic cancer causes has been directly demonstrated by examples in which one tumor-suppressor allele is inactivated by mutations and the other allele is transcriptionally silenced because of hypermethylation (Myohanen et al. 1998; Grady et al. 2000). Also, hypermethylation of tumor-suppressor genes may be an early event in cancer development (Waki et al. 2003; Lee et al. 2004; Suter et al. 2004), suggesting that epigenetic and mutational cancer causes may collaborate from an early time point in disease progression. The list of tumor-suppressor genes found transcriptionally inactivated by hypermethylation in cancer is long and steadily growing (Jones and Baylin 2002), includes genes that are part of every cancer-related pathway, and contains prominent genes such as *CDKN2A* (Herman et al. 1995), *pRB* (Ohtani-Fujita et al. 1993), *APC* (Hiltunen et al. 1997), *PTEN* (Whang et al. 1998), *BRCA1* (Rice et al. 1998), *VHL* (Herman et al. 1994), and *CDH1* (Graff et al. 1995). By extension, epigenetic silencing may underlie genetic cancer causes. Epigenetic induction of a classical mutator phenotype via transcriptional inactivation of the DNA mismatch repair gene *MLH1* has been proposed to account for microsatellite instability in colorectal cancers (Kane et al. 1997; Herman et al. 1998; Toyota et al. 1999a,b), and silencing of the DNA repair gene coding for O6-methylguanine-DNA methyltransferase has been associated with specific mutations in K-RAS (Esteller et al. 2000) and p53 (Nakamura et al. 2001).

In contrast to hypermethylation of tumor-suppressor genes, tumor cells globally display an overall hypomethylation of DNA (Feinberg and Vogelstein 1983a; Goelz et al. 1985) and hypomethylation was the first epigenetic mechanism to be linked to cancer development (Feinberg and Tycko 2004). Global genomic demethylation appears to progress with age in a tissue-dependent manner (Richardson 2003) and may in part explain the higher incidence of cancer among the elderly. Loss of DNA methylation has also been linked to nutrition, as lack of S-adenosylmethione, the primary methyl donor in the cell, has been shown to predispose to cancer (Huang 2002). A decrease in global methylation can be detected prior to tumor formation in rats maintained on a methyldeficient diet (Christman et al. 1993; Pogribny et al. 1997), and hypomethylation has been found to increase the expression of several known oncogenes including CYCLIN D2 (Oshimo et al. 2003), BCL2 (Hanada et al. 1993), and HRAS (Feinberg and Vogelstein 1983b). On a more positive note, global demethylation results in the expression of "cancer and testis-specific" antigens (De Smet et al. 1996; Banchereau et al. 2003). Aside from the diagnostic value of cancer-specific antigens, these proteins constitute potential targets for immunotherapy procedures. Interestingly, microarray analyses have re-

vealed that the aberrant methylation patterns associated with cancer appears to be tumor-type specific (Eads et al. 2000; Esteller et al. 2001; Adorjan et al. 2002; Paz et al. 2003). Genomic hypomethylation may furthermore cause genomic instability, presumably because demethylation predisposes to DNA strand breakage and recombination within derepressed repetitive sequences (for review, see Ehrlich 2002). Indeed, links between hypomethylation and genomic instability have been shown for many cancer types including breast cancer (Tsuda et al. 2002) and prostate cancer (Schulz et al. 2002). Mouse models have further validated a role for genomic hypomethylation in tumor formation, as embryonic stem cells deficient for the Dnmt1 maintenance methyltransferase display an increased frequency of chromosomal rearrangements (Chen et al. 1998), and mice carrying a hypomorph *Dnmt1* allele develop aggressive lymphomas displaying a high frequency of genomic rearrangements (Gaudet et al. 2003).

The underlying mechanisms for the initiation and targeting of ectopic hypermethylation are not known, although it has been suggested that DNMTs may preferentially bind to damaged or mismatched DNA (James et al. 2003). Studies of remethylation of the p16^{INK4A} tumor-suppressor gene following genome-wide demethylation indicates a requirement for DNA replication prior to remethylation (Velicescu et al. 2002). As discussed following, evidence from several model systems has demonstrated that histone methylation imprint may direct DNA methylation (Tamaru and Selker 2001; Bachman et al. 2003). Transcriptional silencing of genes with tumor-suppressive function may also occur via spreading of heterochromatin out from nearby silenced loci of repetitive DNA (Jones and Baylin 2002), and the regulatory regions of many genes (including known tumor suppressors) contain islands of repetitive sequences, some of which have been shown to influence transcriptional regulation (Jordan et al. 2003; Kelly 2003). Spreading of heterochromatin-associated repression may be concurrent with the breakdown of higher-order chromatin structures such as boundary elements. In normal cells, insulator proteins such as the transcription factor CTCF establish chromatin boundaries, and CTCF is involved in the regulation of imprinting, where it is required to protect against de novo methylation (Fedoriw et al. 2004; Lewis and Murrell 2004). Loss of imprinting of the IGF2 gene was recently suggested as a predictive marker for colorectal cancer (Cui et al. 2003). Intriguingly, methylation of the CTCF recognition sequence abolishes CTCF binding (Nakagawa et al. 2001), indicating that specific DNA methylations may have long-range consequences.

In analogy to the mapping of single-nucleotide polymorphisms, large-scale attempts to link epigenetic variation, such as methylation-variable positions, are currently being undertaken (Novik et al. 2002; Fazzari and Greally 2004; e.g., see The Human Epigenome Consortium, http://www.epigenome.org). Such approaches promise to yield valuable tools for future research into links between epigenetics and cancer.

Reading epigenetic codes

Two protein domain families have been described as recognizing epigenetic imprints on histone tails, bromodomain and chromodomain proteins, respectively. Histone modification by acetylation appears to function in part through altering the structural properties of the nucleosome and allowing transcription factors to access the DNA. Also, acetylated lysines serve as binding sites for bromodomain-containing proteins, such as HATs and chromatin remodeling factors, some of which take part in the transcription initiation complex (Dhalluin et al. 1999; Zeng and Zhou 2002; Kanno et al. 2004). Several protein complexes containing a chromodomain (chromatin organization modifier) module have been found capable of reading histone methylation imprints and initiating downstream responses in terms of target gene regulation. More than 25 chromobox proteins are recognizable in the human genome, only a subset of which has been characterized in any detail. In this section, we review cancer connections for prominent "readers" of epigenetic codes.

HP1 proteins

The silencing of heterochromatic regions is in part mediated by the family of heterochromatin-associated proteins, HP1. Three HP1 proteins are found in mammals, where HP1α and HP1β localize to pericentric heterochromatin and minor sites within euchromatin, whereas HP1γ localizes predominantly to euchromatic regions (Eissenberg and Elgin 2000). In both mammals and flies, HP1 acts in a dosage-dependent manner with heterozygotes displaying a partial loss of gene silencing and HP1 overexpression resulting in an increase in silencing (Eissenberg et al. 1992; Festenstein et al. 1999). Whereas the three mammalian HP1 members all hold chromodomains capable of recognizing the methylated Lys 9 on histone H3 (Bannister et al. 2001; Lachner et al. 2001), the differences in subnuclear localizations point to the presence of additional mechanisms for localizing and tethering HP1 proteins. HP1 has been found to interact with many different proteins, spanning from histones over transcriptional regulators to proteins involved in DNA replication and nuclear architecture (for review, see Li et al. 2002). Loss of Suv39h in mice results in the displacement of HP1 proteins from pericentromeric heterochromatin, resulting in anaploidy and cancer (Peters et al. 2001). Few direct links have been established between HP1 expression or localization and cancer, although the expression of HP1α is reportedly down-regulated in invasive human breast cancer cells, and overexpression of HP1α results in diminished invasive potential (Kirschmann et al. 2000). The apparent weak link between HP1 malexpression and cancer development is surprising, given the importance of HP1 proteins in stabilizing critical heterochromatin regions including centromeres. This could be taken to signify the presence of compensatory mechanisms with the capacity to partly substitute for HP1 function. Such compensatory mecha-

nisms were demonstrated recently in mouse ES cells deficient for both Suv39 homologs (Peters et al. 2003). Alternatively, essential roles of HP1 may functionally preclude a complete loss of function, tolerating only subtle fluctuations in levels, which may be difficult to detect in heterologous cancer samples.

Polycomb proteins

The methylation imprint set by EZH2 at Lys 27 on histone H3 mechanistically links the PRC2 and PRC1 PcG complexes, as methylated Lys 27 of histone H3 serves as a binding site for the PRC1 member Pc (Cao et al. 2002; Czermin et al. 2002; Kuzmichev et al. 2002). Bmi1 is the most extensively studied PRC1 member, which has been found to be overexpressed in human tumors (Bea et al. 2001; Vonlanthen et al. 2001; Leung et al. 2004). Originally identified as a potent collaborator with Myc in mouse leukemia (van Lohuizen et al. 1991), Bmi1 was subsequently demonstrated to exert oncogenic effects through down-regulation of the CDK inhibitors p16^{ink4a} and p19^{Arf}, thereby impinging on both the pRB and the p53 pathways (Jacobs et al. 1999a,b). In cell culture models, BMI1 regulates replicative senescence in human fibroblasts and mammary epithelial cells via repression of p16 and indirectly via induction of hTert (Dimri et al. 2002; Itahana et al. 2003). Edr1, another PRC1 member, has been reported to be required for sustained hematopoiesis in the mouse (Ohta et al. 2002). Recently, a new PRC1 member, Cbx7, was identified in a genetic screen for bypass of cellular senescence and shown to suppress the *Ink4a* tumor-suppressor locus independent of Bmi1 (Gil et al. 2004). In addition, individual PcG members have been identified in several protein complexes central to cell cycle regulation (Dahiya et al. 2001; Ogawa et al. 2002; Shi et al. 2003). Hence, ample evidence exists connecting PcG-mediated epigenetic repression to cancer development. Recently, additional cancer-related functions of Bmi1 were elucidated, as Bmi1 proved indispensable for the maintenance of hematopoietic and leukemic stem cells (Lessard and Sauvageau 2003; Park et al. 2003) and neuronal stem cell populations (Molofsky et al. 2003; Leung et al. 2004). These studies place Bmi1, and thereby epigenetic gene regulation, central to the emerging concept of tumor stem cells, which aside from having escaped cellular growth control mechanisms such as the p53 and the pRB pathways, also have (re)instigated a stem cell-like transcription program that allows for continuous self-renewal (Pardal et al. 2003; Valk-Lingbeek et al. 2004).

Methyl-DNA-binding proteins

Why is methylated DNA transcriptionally silent? A few cases have been described in which DNA-binding proteins are unable to bind their cognate DNA recognition sequence because of steric hindrance from an attached methyl group (Nakagawa et al. 2001). A more predominant mechanism is mediated via a group of proteins with the capacity to read the epigenetic methyl-CpG code: the methyl-CpG-binding proteins (Bird 2002). Thus far, six methyl-binding proteins have been described. MBD1, MBD2, MBD3, MeCP2, and KAISO are all involved in transcriptional repression, whereas MBD4 functions in DNA mismatch repair. Methyl-CpG-binding proteins associate with histone modifying enzymes to maintain transcriptional silence, and methyl-DNA-binding proteins have been found associated with aberrantly methylated promoter regions of cancer-relevant genes such as p16CDKN2A (Nguyen et al. 2001) and MGMT (Nakagawachi et al. 2003). Although MeCP2, MBD2, and MBD4 have been found down-regulated in human cancers (Zhu et al. 2004b), perhaps surprisingly, no firm cancer connections have been established for any of the methyl-binding proteins. MBD1 and MBD2 reside on human chromosome 18q21, a region frequently lost in cancer, but mutation analysis from human lung and colon cancers revealed few changes in MBD1 or MBD2, indicating a limited role for these proteins in cancer (Bader et al. 2003). By extension, mouse knockout studies have not demonstrated important tumor-suppressor functions for Mecp2, although long-term studies have been precluded, as these mice succumb early in life to neurological disorders (Chen et al. 2001; Guy et al. 2001). In both mice and humans, mutations in *MECP2* cause the neurodevelopmental disorder Rett syndrome; however, patients with Rett syndrome do not appear to be predisposed to cancer development (Amir et al. 1999; Chen et al. 2001; Guy et al. 2001). Also, Mbd2 deficiency does not predispose to tumor formation. On the contrary, when bred onto a bona fide model for intestinal cancer, the *Apc*^{min} mouse, loss of Mbd2 significantly delays tumorigenesis (Sansom et al. 2003). Furthermore, MBD2 knockdown in human cancer cell lines was found to suppress tumorigenesis in a mouse xenograft model (Ivanov et al. 2003; Campbell et al. 2004). Being the functional interpreters of DNA methylation, prominent roles for methyl-DNA-binding proteins in cancer could be envisioned. The lack evidence supporting this could reflect the two-faced nature of DNA methylation: promoting oncogenesis via tumor-suppressor hypermethylation and protecting genome integrity through repression of repetitive DNA. Also, additional methyl-binding proteins lacking the classical methyl-binding domain may yet be discovered.

Messing around with nucleosomes

Nucleosome remodeling complexes modify chromatin topology in an ATP-dependent manner by disrupting DNA:histone interactions, thereby facilitating sliding of the nucleosome, and hence the accessibility of the DNA to transcription factors (Becker and Horz 2002).

The SWI/SNF complex regulates genes locally, and analyses of yeast SWI/SNF mutants revealed that transcription of ~5% of all yeast genes is influenced by SWI/SNF mutations (Sudarsanam et al. 2000). The SWI/SNF core complex consists of SNF5/INI1, BRG1, BRM, BAF155, and BAF170. SWI/SNF interacts with many protein complexes central to cancer development, such as

RB, p53, MYC, MLL, BRCA1, and β-catenin; hence, functional inactivation of SWI/SNF impinges on a multitude of cellular growth control pathways (for a recent review, see Roberts and Orkin 2004).

Most compelling, analyses of the SWI/SNF core subunit SNF5 (INI1) have revealed the presence of inactivating mutations in highly aggressive human malignant rhabdoid tumors (Versteege et al. 1998; Biegel et al. 1999). SNF5 mutations are underlying familial cancers in which one SNF5 allele carries a germ-line mutation and the other allele is lost during tumorigenesis (Versteege et al. 1998; Sevenet et al. 1999; Taylor et al. 2000). The tumor-suppressive effect of SNF5 is evident from the fact that reintroduction of SNF5 into SNF5-mutant tumor cells mediates cell cycle arrest (Betz et al. 2002; Reincke et al. 2003) and from mouse models demonstrating *Snf5* as a haploinsufficient tumor-suppressor gene (Klochendler-Yeivin et al. 2000; Roberts et al. 2000), complete loss of which causes mice to succumb early in life to aggressive lymphomas or rhabdoid tumors (Roberts et al. 2002).

BRM and BRG1 are ATPase core subunits of the mammalian SWI/SNF complex. The two proteins are 75% similar in protein composition, are mutually exclusive in chromatin remodeling complexes in vitro (Phelan et al. 1999), and appear to have tumor-suppressor functions. BRG1 has been found mutated in cell lines from lung, pancreas, prostate, and breast cancers (Wong et al. 2000; Decristofaro et al. 2001), and BRG1/BRM expression was found lost in 10% of primary lung tumors correlating with a poor prognostic outcome (Reisman et al. 2003). Whereas Brg1-deficiency causes early embryonic lethality in mice, Brg1$^{+/-}$ animals are prone to epithelial tumors, possibly due to haploinsufficiency for Brg1 in tumor suppression, as the outgrowing tumors retained the remaining wild-type Brg1 allele (Bultman et al. 2000). Brm-deficient mice are viable, likely via adaptive upregulation of Brg1 (Reyes et al. 1998). Although Brm$^{-/-}$ mice do not appear tumor prone, the mice are larger than wild type, and isolated mutant fibroblast cells display G0/G1 checkpoint failure on DNA damage, indicating a role for BRM in cell cycle regulation (Reyes et al. 1998). As BRM was originally identified in *Drosophila* as a suppressor of Polycomb (Tamkun et al. 1992), it is tempting to speculate that loss of BRM or BRG1 impinge on cellular homeostasis by affecting the balance between TrxG and PcG protein complexes.

Variant histones

Currently, dynamic deposition of variant histone proteins is receiving increased attention. In contrast to canonical histones synthesized and deposited during S-phase, variant histones can be synthesized between S-phases and deposited in a dynamic manner (for review, see Henikoff et al. 2004). Recent data suggest that deposited variant histones may represent an additional layer of epigenetic regulation. The H2A variant H2AX marks DNA double-strand breaks, and H2AX-deficient cells have increased genomic instability and impaired formation of radiation-induced foci of DNA repair proteins such as BRCA1 (Bassing et al. 2002; Celeste et al. 2002). Loss of H2AX results in a G2-M checkpoint defect similar to that observed in ATM-deficient cells (Fernandez-Capetillo et al. 2002). H2AX-deficient mice are predisposed to lymphomagenesis, a condition dramatically increased by loss of p53 (Bassing et al. 2003; Celeste et al. 2003). H2AX therefore appears to constitute a bona fide epigenetic tumor suppressor mechanism.

In centromeric chromatin, CENP-A substitutes for histone H3 in the nucleosome (Yoda et al. 2000), and CENP-A was recently found to be overexpressed and mislocalized in primary human colorectal tumors, suggesting a link between CENP-A deregulation and aneuploidy (Tomonaga et al. 2003). Other histone variants directly influence transcriptional activity. In yeast, deposition of H2AZ acts to prevent the binding of silencing proteins to regions of euchromatin (Meneghini et al. 2003), and in mammalian cells H2AZ is necessary for chromosome segregation and correct deposition of HP1α (Rangasamy et al. 2004). Likewise, the presence of macroH2A correlates with transcriptional repression, possibly by precluding the access of transcription factors to the DNA and impeding the function of nucleosome remodeling factors (Angelov et al. 2003). The human genome encodes many more variant histone proteins, only a few of which have been characterized in detail. Some are likely epigenetic regulators of gene expression and thus likely candidates to be captured in neoplastic disorders.

RNA epigenetics and cancer

The significance of noncoding RNAs in processes such as chromatin dynamics and gene silencing has received increased attention over the last years, especially following the unmasking of the large group of small regulatory microRNAs (Bartel 2004). Noncoding RNAs have long been known to regulate fundamental processes, such as the function of *Xist* in the initiation of X-chromosome inactivation (Plath et al. 2002); also, processes such as Polycomb-mediated silencing (Pal-Bhadra et al. 2002) and the association of HP1 to chromatin have been reported to involve an RNA moiety (Maison et al. 2002). In addition, noncoding antisense RNAs are involved in processes of imprinting (Rougeulle and Heard 2002; Sleutels et al. 2002), suggesting a pattern in which antisense RNA can induce transcriptional silencing as a means of gene regulation in normal and disease cells. This theory was recently supported for the α-globin gene from a patient carrying a deletion that juxtaposes the highly expressed *LUC7L* gene in antisense to α-globin (Tufarelli et al. 2003). Importantly, the antisense RNA induces transcriptional silencing of the α-globin gene associated with methylation of the CpG island at the α-globin gene. Also, data from fission yeast implicate the RNAi machinery in the initiation and maintenance of heterochromatin (Hall et al. 2002; Volpe et al. 2002).

MicroRNAs are small noncoding RNAs that regulate gene expression by posttranscriptional mechanisms and

many microRNAs could be prime suspects for cancer promoters because, on the basis of computer predictions, they have been proposed to regulate many cell cycle control genes (Lewis et al. 2003). Interestingly, the precursor miR155/*BIC* was recently reported to be up-regulated in cells of Hodgkin lymphoma (van den Berg et al. 2003). *Bic* was originally identified by retroviral insertional mutagenesis in chickens, as a locus specifically activated by viral insertions and collaborating with *c-myc* in lymphomagenesis (Tam et al. 1997, 2002). Although the causal relationship has not yet been tested in a suitable animal model, miR-155 appears to be a prime candidate for a microRNA acting as a proto-oncogene. The microRNA genes miR15 and miR16 are reportedly down-regulated in two-thirds of analyzed cases of chronic lymphocytic leukemia (Calin et al. 2002), and a genome-wide survey of 186 human microRNAs genes has revealed that microRNAs genes are nonrandomly distributed in the genome and frequently locate to known fragile sites and loci involved in cancer (Calin et al. 2004). Furthermore, small noncoding RNAs are involved in the silencing of repetitive DNA elements, such as retrotransposons (Schramke and Allshire 2003), and in the nucleation of heterochromatin silencing (Reinhart and Bartel 2002; Pal-Bhadra et al. 2004; for a recent review, see Grewal and Rice 2004) and may thereby be important for genome stability and integrity. Conceivably, the emerging connections between the RNAi machinery and gene regulation will reveal fundamental biological knowledge of importance for the understanding of both normal and neoplastic cells.

Stability and dynamics of epigenetic gene regulation

For most loci, epigenetic gene regulation is characterized by a high degree of constancy and stability, ensuring that cells remain in a correct stage of differentiation. How then is this stability achieved and maintained? The mechanism for semiconservative inheritance of DNA methylation is well described. DNMT1 associates with the replication machinery and, being a hemi-methylase, recognizes one-sided methylation of the original strand in CpG palindromes and consequently methylates the newly synthesized complementary strand. Also, the balance between gene activation induced by TrxG proteins and PcG-mediated gene repression is known to be stably inherited, even through meiosis (Cavalli and Paro 1998). The molecular basis for how the epigenetic information carried in histone tail modifications is memorized is unknown. Interestingly, biochemical data have suggested the histones H3 and H4 to be deposited into nascent nucleosomes as heterodimers (Tagami et al. 2004). This opens the possibility that the existing epigenetically coded H3/H4 dimers are divided on the two daughter strands, thereby forming the basis for an epigenetic memory imprint.

For historical reasons, much of the research in epigenetic gene regulation has focused on mechanisms for maintenance of stable gene repression, such as DNA methylation, the Suv39-Hp1, and the PcG system. Often

these mechanisms have been portrayed as static suppressors invoked to ensure long-term transcriptional silencing. This view has recently been challenged on several fronts with evidence of highly dynamic regulation and lively complex reassembly. For instance, recent data suggest important functions outside heterochromatin and point to Suv39-independent functions of HP1 members and vice versa (Greil et al. 2003). Further more, photobleaching experiments using fluorochrome-tagged HP1 demonstrated that the majority of the HP1 proteins are highly mobile with rapid movements in and out of heterochromatin domains (Festenstein et al. 2003; Schmiedeberg et al. 2004).

Several studies have implicated epigenetic regulators in dynamic tumor-suppressor complexes. Aside from interactions between RB and HDACs, the RB protein has been shown to interact with PcG proteins (Dahiya et al. 2001) and to associate with HP1 and SUV39H1 to direct histone methylation to target promoters (Nielsen et al. 2001; Narita et al. 2003). As mentioned earlier, aside from SUV39H, RB also associates with the RIZ HMT (Buyse et al. 1995). Epigenetic setting can also be dynamically affected by external stimuli. This is well documented in plants where vernalization processes are sensitive to temperature shifts. Vernalization is controlled by the FLC protein, expression of which is influenced by changes in DNA methylation and histone methylation status in a temperature-dependent manner, conferring to the plant an epigenetic memory of winter (Bastow et al. 2004; Sung and Amasino 2004). Also, recent work on the regulation of brain-derived neurotrophic factor (BDNF) underlines the short-term dynamic properties of epigenetic gene regulation. BDNF is synthesized in response to neuronal stimuli and has been implicated in a variety of neuronal processes including learning and memory. Interestingly, two groups showed that on stimulation of cultured neurons, induction of BDNF expression is associated with a decrease in CpG methylation at the BDNF promoter and release of the MeCP2 repressive complex (Chen et al. 2003; Martinowich et al. 2003). This work challenges the notion of DNA methylation as a static mechanism for long-term gene silencing.

Integrative epigenetics: forces of stability

Transcriptional inactivation can follow several routes and ample evidence proves biochemical associations between different epigenetic layers en route to epigenetic silencing: (1) DNA methylation may dictate histone modification, (2) histone modification may mediate DNA methylation, and (3) nucleosome remodeling may facilitate DNA methylation.

DNA methylation can affect histone modification patterns, as DNMT enzymes directly recruit both deacetylase and methyltransferase activity to mediate transcriptional silence (Fig. 3) (Fuks et al. 2000; Robertson et al. 2000; Rountree et al. 2000). In extension, CpG-methylated DNA associates with methyl-CpG-binding proteins, such as MeCP2 and MBD2, which in turn complex with histone modifiers like HDACs (Jones et al. 1998;
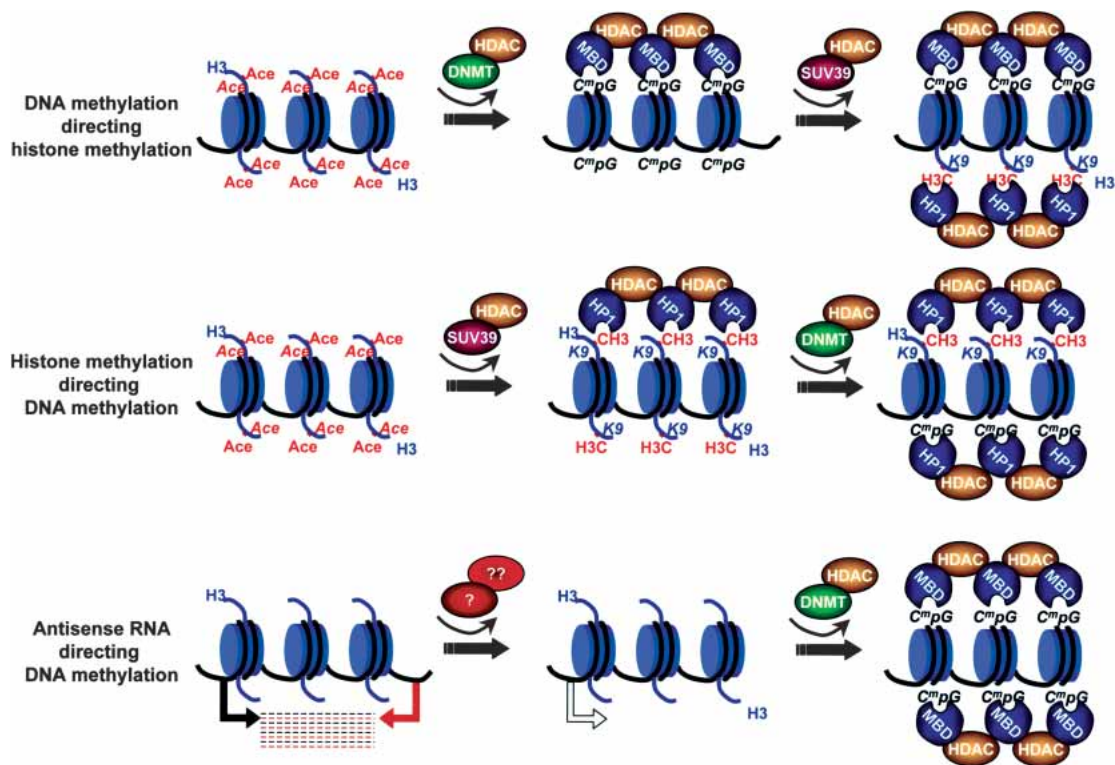
Figure 3. Integrative epigenetic gene repression. (*Top*) DNA methylation driving histone modification. (*Middle*) Histone modification directing DNA methylation. (*Bottom*) Noncoding antisense RNA driving DNA methylation. See text for details.

Nan et al. 1998; Wade et al. 1999) and HMTs (Fuks et al. 2003), thereby cementing transcriptional repression. Several lines of genetic and biochemical evidence suggest that histone modification may precede and promote DNA methylation. This is evident during inactivation of the redundant X-chromosome in female mammals, where methylation of histone H3 Lys 9 and Lys 27 precedes DNA methylation (Okamoto et al. 2004). Genetic links between histone modification and DNA methylation have been demonstrated in the fungus *Neurospora crassa*, where mutation of the histone H3 Lys 9-specific methyltransferase abolishes cytosine methylation (Tamaru and Selker 2001).

Also, methylation of histone H3 Lys 9 enables binding of HP1 proteins, which in turn can recruit DNMTs (Lehnertz et al. 2003). Finally, nucleosome remodeling can affect DNA methylation, as evident from genetic studies in *Arabidopsis thaliana*, where mutation of the SNF2-like gene *DDM1* causes a dramatic decrease in the level of genomic cytosine methylation (Jeddeloh et al. 1999). In the mouse, disruption of the *Lsh* locus encoding the SNF2-like helicase PASG results in genomic demethylation (Dennis et al. 2001; Sun et al. 2004), demonstrating concerted functionality between nucleosome remodeling and DNA methylation. Lsh deficiency also affects histone methylation patterns at pericentromeric heterochromatin, resulting in the accumulation of methylated Lys 4 of histone H3 and pointing to cross-talk between epigenetic layers of regulation (Yan et al. 2003).

Interestingly, abrogation of the *lsh* locus results in a dramatic induction of the cyclin-dependent kinase inhibitor p16[Ink4a] (Sun et al. 2004). This effect is independent of p16[Ink4a] promoter methylation but could in part be explained by reduced levels of the known p16[Ink4a] regulator Polycomb protein Bmi1 in *lsh* knockouts (Sun et al. 2004). Although factors other than Bmi1 are likely involved, this example illustrates the multilayered nature of epigenetic gene regulation and points to PASG as an upstream regulator of several epigenetic machineries.

Integrative epigenetics: the Achilles' heel

Although the integrative cooperativity between epigenetic mechanisms acts to ensure proper gene expression in the healthy cell, the same functional interlocking is captured in some cancers to instigate aberrant gene expressions.

From hematopoietic cancers, several specific translocations have been characterized, in which chimeric fusion oncoproteins exhibit the opposite function from the wild-type protein or have lost important regulatory features. Clear examples follow. The AML1 gene (also known as CBFA2 and RUNX1) is required for differentiation of hematopoietic cells and can act as a gene activator or repressor dependent on the association with either the corepressor Groucho or the HAT complex CBP/p300. The relatively common t(8;21) translocation fuses the DNA-binding domain of AML1 to ETO (eight-

twenty-one), which in turn associates with gene repressors such as HDACs and Sin3. The AML1–ETO fusion suppresses AML1 target genes, leading to a block in myeloid differentiation and cellular transformation (for review, see Peterson and Zhang 2004). Likewise, the numerous translocations involving MLL1 likely promotes leukemia through messing up epigenetic codes. Two translocation events involving the retinoic acid receptor-α have been characterized in some detail: PML–RARα and PLZR–RARα. The leukemia-promoting PML–RARα (promyelocytic leukemia) fusion protein induces hypermethylation and silencing of RARα target genes via the recruitment of HDACs and DNMTase activity (Di Croce et al. 2002). The induced hypermethylation is linked to oncogenesis and treatment with retinoic acid reverses the transformed phenotype through the induction of target gene demethylation and re-expression. The promyelocytic leukemia zinc finger (PLZR) protein also associates with transcriptional repressors such as HDACs and PcG proteins (Barna et al. 2002) and the PLZR–RARα fusion likewise represses genes normally activated by RARα. In contrast to PML–RARα fusions, PLZR–RARα target genes are insensitive to retinoic acid treatments, despite the fact that retinoic acid does induce PLZR–RARα degradation (Rego et al. 2000).

Conclusions

From a larger perspective, two phenomena demonstrate the significance of epigenetics in cancer development. First, the influence of epigenetics on tumor development is reflected in the importance of extracellular matrix signaling and tumor cell–stromal cell interactions. Notwithstanding the importance of genetic alterations enabling tumor cells to escape defense mechanisms such as the p53 and pRB pathways and the "telomere clock", malignant transformation is highly dependent on the surroundings for successful outgrowth. Hence, the tumor microenvironment may itself be viewed as an epigenetic modifier with the potential to promote or prevent malignant outgrowth (Hanahan and Weinberg 2000; Weaver and Gilbert 2004).

Second, directly proving the importance of epigenetics in tumorigenic processes are the emerging successful treatments of cancers with inhibitors of epigenetic regulators. Many chemical agents have been discovered to selectively inhibit either DNMTs or HDACs, and several of these compounds are currently going through clinical trials (Claus and Lubbert 2003; Egger et al. 2004). The fundamental principle behind this type of epigenetic therapy is that reversal of epigenetic silencing will reinstate cellular cancer defense mechanisms, for instance via induced expression of the cyclin-dependent kinase inhibitors p16 and p21. Gene hypermethylation and histone hypoacetylation are attractive targets for the treatment of epigenetic diseases and differ in their intrinsic reversibility from diseases founded in genetic alterations such as translocations and mutations. Inhibitors of DNMTs and HDACs affect cells on a global scale and combination treatments using both drug types have

proven very useful, partly because of increased efficiency after therapeutic targeting of independent epilayers, and partly because combination treatments allow for the usage of lower doses of the drugs with fewer side effects (Egger et al. 2004). In many cancers, several tumor-suppressor pathways have become inactivated, and epigenetic therapy offers the potential of targeting several genes with one drug. The other side of that coin is that many "innocent bystander genes" are likely to be affected and, as yet, relatively little is known about potential side effects in patients.

The significance of epigenetic mechanisms for gene regulation in cancer is now evident with cancer-related mechanisms acting at all epilayers. The study of epigenetic imprints is still in its infancy, as not all epigenetic marks are known, and only few of the known ones are understood in any detail. Hence, the near future is likely to bring important new insights into epigenetic gene regulation in both normal and neoplastic development as high-quality tools such as methyl-specific histone antibodies and new chromatin exploration methodologies emerge.

References

Adorjan, P., Distler, J., Lipscher, E., Model, F., Muller, J., Pelet, C., Braun, A., Florl, A.R., Gutig, D., Grabs, G., et al. 2002. Tumour class prediction and discovery by microarray-based DNA methylation analysis. *Nucleic Acids Res.* **30:** e21.

Amir, R.E., Van den Veyver, I., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. 1999. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* **23:** 185–188.

Angelov, D., Molla, A., Perche, P.Y., Hans, F., Cote, J., Khochbin, S., Bouvet, P., and Dimitrov, S. 2003. The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling. *Mol. Cell* **11:** 1033–1041.

Antequera, F. and Bird, A. 1993. Number of CpG islands and genes in human and mouse. *Proc. Natl. Acad. Sci.* **90:** 11995–11999.

Arakawa, H., Nakamura, T., Zhadanov, A.B., Fidanza, V., Yano, T., Bullrich, F., Shimizu, M., Blechman, J., Mazo, A., Canaani, E., et al. 1998. Identification and characterization of the ARP1 gene, a target for the human acute leukemia ALL1 gene. *Proc. Natl. Acad. Sci.* **95:** 4573–4578.

Ayton, P.M. and Cleary, M.L. 2001. Molecular mechanisms of leukemogenesis mediated by MLL fusion proteins. *Oncogene* **20:** 5695–5707.

———. 2003. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes & Dev.* **17:** 2298–2307.

Bachman, K.E., Rountree, M.R., and Baylin, S.B. 2001. Dnmt3a and Dnmt3b are transcriptional repressors that exhibit unique localization properties to heterochromatin. *J. Biol. Chem.* **276:** 32282–32287.

Bachman, K.E., Park, B.H., Rhee, I., Rajagopalan, H., Herman, J.G., Baylin, S.B., Kinzler, K.W., and Vogelstein, B. 2003. Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. *Cancer Cell* **3:** 89–95.

Bader, S., Walker, M., McQueen, H.A., Sellar, R., Oei, E., Wopereis, S., Zhu, Y., Peter, A., Bird, A.P., and Harrison, D.J. 2003. MBD1, MBD2 and CGBP genes at chromosome 18q21

are infrequently mutated in human colon and lung cancers. *Oncogene* **22:** 3506–3510.

Bakin, A.V. and Curran, T. 1999. Role of DNA 5-methylcytosine transferase in cell transformation by fos. *Science* **283:** 387–390.

Banchereau, J., Fay, J., Pascual, V., and Palucka, A.K. 2003. Dendritic cells: Controllers of the immune system and a new promise for immunotherapy. *Novartis Found. Symp.* **252:** 226–235.

Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C., and Kouzarides, T. 2001. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410:** 120–124.

Barjesteh van Waalwijk van Doorn-Khosrovani, S., Erpelinck, C., van Putten, W.L., Valk, P.J., van der Poel-van de Luytgaarde, S., Hack, R., Slater, R., Smit, E.M., Beverloo, H.B., Verhoef, G., et al. 2003. High EVI1 expression predicts poor survival in acute myeloid leukemia: A study of 319 de novo AML patients. *Blood* **101:** 837–845.

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.

Bartel, D.P. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* **116:** 281–297.

Bassing, C.H., Chua, K.F., Sekiguchi, J., Suh, H., Whitlow, S.R., Fleming, J.C., Monroe, B.C., Ciccone, D.N., Yan, C., Vlasakova, K., et al. 2002. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proc. Natl. Acad. Sci.* **99:** 8173–8178.

Bassing, C.H., Suh, H., Ferguson, D.O., Chua, K.F., Manis, J., Eckersdorff, M., Gleason, M., Bronson, R., Lee, C., and Alt, F.W. 2003. Histone H2AX: A dosage-dependent suppressor of oncogenic translocations and tumors. *Cell* **114:** 359–370.

Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., and Dean, C. 2004. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* **427:** 164–167.

Bea, S., Tort, F., Pinyol, M., Puig, X., Hernandez, L., Hernandez, S., Fernandez, P.L., van Lohuizen, M., Colomer, D., and Campo, D. 2001. BMI-1 gene amplification and overexpression in hematological malignancies occur mainly in mantle cell lymphomas. *Cancer Res.* **61:** 2409–2412.

Beaulieu, N., Morin, S., Chute, I.C., Robert, M.F., Nguyen, H., and MacLeod, A.R. 2002. An essential role for DNA methyltransferase DNMT3B in cancer cell survival. *J. Biol. Chem.* **277:** 28176–28181.

Becker, P.B. and Horz, W. 2002. ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **71:** 247–273.

Beisel, C., Imhof, A., Greene, J., Kremmer, E., and Sauer, F. 2002. Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. *Nature* **419:** 857–862.

Bergsagel, P.L. and Kuehl, W.M. 2001. Chromosome translocations in multiple myeloma. *Oncogene* **20:** 5611–5622.

Betz, B.L., Strobeck, M.W., Reisman, D.N., Knudsen, E.S., and Weissman, B.E. 2002. Re-expression of hSNF5/INI1/BAF47 in pediatric tumor cells leads to G1 arrest associated with induction of p16ink4a and activation of RB. *Oncogene* **21:** 5193–5203.

Biegel, J.A., Zhou, J.Y., Rorke, L.B., Stenstrom, C., Wainwright, L.M., and Fogelgren, B. 1999. Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. *Cancer Res.* **59:** 74–79.

Bird, A. 2002. DNA methylation patterns and epigenetic memory. *Genes & Dev.* **16:** 6–21.

Borrow, J., Stanton Jr., V.P., Andresen, J.M., Becher, R., Behm, F.G., Chaganti, R.S., Civin, C.I., Disteche, C., Dube, I., Frischauf, A.M., et al. 1996. The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nat. Genet.* **14:** 33–41.

Bracken, A.P., Pasini, D., Capra, M., Prosperini, E., Colli, E., and Helin, K. 2003. EZH2 is downstream of the pRB–E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.* **22:** 5323–5335.

Bultman, S., Gebuhr, T., Yee, D., La Mantia, C., Nicholson, J., Gilliam, A., Randazzo, F., Metzger, D., Chambon, P., Crabtree, G., et al. 2000. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol. Cell* **6:** 1287–1295.

Buyse, I.M., Shao, G., and Huang, S. 1995. The retinoblastoma protein binds to RIZ, a zinc-finger protein that shares an epitope with the adenovirus E1A protein. *Proc. Natl. Acad. Sci.* **92:** 4467–4471.

Buyse, I.M., Takahashi, E.I., and Huang, S. 1996. Physical mapping of the retinoblastoma interacting zinc finger gene RIZ to D1S228 on chromosome 1p36. *Genomics* **34:** 119–121.

Calin, G.A., Dumitru, C.D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Aldler, H., Rattan, S., Keating, M., Rai, K., et al. 2002. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci.* **99:** 15524–15529.

Calin, G.A., Sevignani, C., Dumitru, C.D., Hyslop, T., Noch, E., Yendamuri, S., Shimizu, M., Rattan, S., Bullrich, F., Negrini, M., et al. 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proc. Natl. Acad. Sci.* **101:** 2999–3004.

Campbell, P.M., Bovenzi, V., and Szyf, M. 2004. Methylated DNA-binding protein 2 antisense inhibitors suppress tumourigenesis of human cancer cell lines in vitro and in vivo. *Carcinogenesis* **25:** 499–507.

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:** 1039–1043.

Caron, C., Col, E., and Khochbin, S. 2003. The viral control of cellular acetylation signaling. *Bioessays* **25:** 58–65.

Cavalli, G. and Paro, R. 1998. The *Drosophila* Fab-7 chromosomal element conveys epigenetic inheritance during mitosis and meiosis. *Cell* **93:** 505–518.

Celeste, A., Petersen, S., Romanienko, P.J., Fernandez-Capetillo, O., Chen, H.T., Sedelnikova, O.A., Reina-San-Martin, B., Coppola, V., Meffre, E., Difilippantonio, M.J., et al. 2002. Genomic instability in mice lacking histone H2AX. *Science* **296:** 922–927.

Celeste, A., Difilippantonio, S., Difilippantonio, M.J., Fernandez-Capetillo, O., Pilch, D.R., Sedelnikova, O.A., Eckhaus, M., Ried, T., Bonner, W.M., and Nussenzweig, A. 2003. H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. *Cell* **114:** 371–383.

Chadwick, R.B., Jiang, G.L., Bennington, G.A., Yuan, B., Johnson, C.K., Stevens, M.W., Niemann, T.H., Peltomaki, P., Huang, S., and de la Chapelle, A. 2000. Candidate tumor suppressor RIZ is frequently involved in colorectal carcinogenesis. *Proc. Natl. Acad. Sci.* **97:** 2662–2667.

Chen, R.Z., Pettersson, U., Beard, C., Jackson-Grusby, L., and Jaenisch, R. 1998. DNA hypomethylation leads to elevated mutation rates. *Nature* **395:** 89–93.

Chen, R.Z., Akbarian, S., Tudor, M., and Jaenisch, R. 2001. Deficiency of methyl-CpG binding protein-2 in CNS neurons

results in a Rett-like phenotype in mice. *Nat. Genet.* **27:** 327–331.

Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R., and Greenberg, M.E. 2003. Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* **302:** 885–889.

Cheung, P., Tanner, K.G., Cheung, W.L., Sassone-Corsi, P., Denu, J.M., and Allis, C.D. 2000. Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation. *Mol. Cell* **5:** 905–915.

Christman, J.K., Sheikhnejad, G., Dizik, M., Abileah, S., and Wainfan, E. 1993. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis* **14:** 551–557.

Claus, R. and Lubbert, M. 2003. Epigenetic targets in hematopoietic malignancies. *Oncogene* **22:** 6489–6496.

Corral, J., Lavenir, I., Impey, H., Warren, A.J., Forster, A., Larson, T.A., Bell, S., McKenzie, A.N., King, G., and Rabbitts, T.H. 1996. An Mll–AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: A method to create fusion oncogenes. *Cell* **85:** 853–861.

Cui, H., Cruz-Correa, M., Giardiello, F.M., Hutcheon, D.F., Kafonek, D.R., Brandenburg, S., Wu, Y., He, X., Powe, N.R., and Feinberg, A.P. 2003. Loss of IGF2 imprinting: A potential marker of colorectal cancer risk. *Science* **299:** 1753–1755.

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:** 185–196.

Dahiya, A., Wong, S., Gonzalo, S., Gavin, M., and Dean, D.C. 2001. Linking the Rb and polycomb pathways. *Mol. Cell* **8:** 557–569.

Daujat, S., Bauer, U.M., Shah, V., Turner, B., Berger, S., and Kouzarides, T. 2002. Crosstalk between CARM1 methylation and CBP acetylation on histone H3. *Curr. Biol.* **12:** 2090–2097.

De Marzo, A.M., Marchi, V.L., Yang, E.S., Veeraswamy, R., Lin, X., and Nelson, W.G. 1999. Abnormal regulation of DNA methyltransferase expression during colorectal carcinogenesis. *Cancer Res.* **59:** 3855–3860.

De Smet, C., De Backer, O., Faraoni, I., Lurquin, C., Brasseur, F., and Boon, T. 1996. The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. *Proc. Natl. Acad. Sci.* **93:** 7149–7153.

Decristofaro, M.F., Betz, B.L., Rorie, C.J., Reisman, D.N., Wang, W., and Weissman, B.E. 2001. Characterization of SWI/SNF protein expression in human breast cancer cell lines and other malignancies. *J. Cell. Physiol.* **186:** 136–145.

Dennis, K., Fan, T., Geiman, T., Yan, Q., and Muegge, K. 2001. Lsh, a member of the SNF2 family, is required for genome-wide methylation. *Genes* & *Dev.* **15:** 2940–2944.

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:** 491–496.

Di Croce, L., Raker, V.A., Corsaro, M., Fazi, F., Fanelli, M., Faretta, M., Fuks, F., Lo, C.F., Kouzarides, T., Nervi, C., et al. 2002. Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* **295:** 1079–1082.

Dimri, G.P., Martinez, J.L., Jacobs, J.J., Keblusek, P., Itahana, K., van Lohuizen, M., Campisi, J., Wazer, D.E., and Band, V. 2002. The Bmi-1 oncogene induces telomerase activity and immortalizes human mammary epithelial cells. *Cancer Res.*

62: 4736–4745.

Djabali, M., Selleri, L., Parry, P., Bower, M., Young, B.D., and Evans, G.A. 1992. A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukaemias. *Nat. Genet.* **2:** 113–118.

Dobson, C.L., Warren, A.J., Pannell, R., Forster, A., Lavenir, I., Corral, J., Smith, A.J., and Rabbitts, T.H. 1999. The mll–AF9 gene fusion in mice controls myeloproliferation and specifies acute myeloid leukaemogenesis. *EMBO J.* **18:** 3564–3574.

Du, Y., Carling, T., Fang, W., Piao, Z., Sheu, J.C., and Huang, S. 2001. Hypermethylation in human cancers of the RIZ1 tumor suppressor gene, a member of a histone/protein methyltransferase superfamily. *Cancer Res.* **61:** 8094–8099.

Eads, C.A., Danenberg, K.D., Kawakami, K., Saltz, L.B., Blake, C., Shibata, D., Danenberg, P.V., and Laird, P.W. 2000. MethyLight: A high-throughput assay to measure DNA methylation. *Nucleic Acids Res.* **28:** E32.

Egger, G., Liang, G., Aparicio, A., and Jones, P.A. 2004. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* **429:** 457–463.

Ehrlich, M. 2002. DNA methylation in cancer: Too much, but also too little. *Oncogene* **21:** 5400–5413.

Eissenberg, J.C. and Elgin, S.C. 2000. The HP1 protein family: Getting a grip on chromatin. *Curr. Opin. Genet. Dev.* **10:** 204–210.

Eissenberg, J.C., Morris, G.D., Reuter, G., and Hartnett, T. 1992. The heterochromatin-associated protein HP-1 is an essential protein in *Drosophila* with dosage-dependent effects on position-effect variegation. *Genetics* **131:** 345–352.

Ernst, P., Wang, J., Huang, M., Goodman, R.H., and Korsmeyer, S.J. 2001. MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Mol. Cell. Biol.* **21:** 2249–2258.

Esteller, M., Toyota, M., Sanchez-Cespedes, M., Capella, G., Peinado, M.A., Watkins, D.N., Issa, J.P., Sidransky, D., Baylin, S.B., and Herman, J.G. 2000. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. *Cancer Res.* **60:** 2368–2371.

Esteller, M., Corn, P.G., Baylin, S.B., and Herman, J.G. 2001. A gene hypermethylation profile of human cancer. *Cancer Res.* **61:** 3225–3229.

Fabbrizio, E., El Messaoudi, S., Polanowska, J., Paul, C., Cook, J.R., Lee, J.H., Negre, V., Rousset, M., Pestka, S., Le Cam, A., et al. 2002. Negative regulation of transcription by the type II arginine methyltransferase PRMT5. *EMBO Rep.* **3:** 641–645.

Fazzari, M.J. and Greally, J.M. 2004. Epigenomics: Beyond CpG islands. *Nat. Rev. Genet.* **5:** 446–455.

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:** 238–240.

Feinberg, A.P. and Tycko, B. 2004. The history of cancer epigenetics. *Nat. Rev. Cancer* **4:** 143–153.

Feinberg, A.P. and Vogelstein, B. 1983a. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* **301:** 89–92.

———. 1983b. Hypomethylation of ras oncogenes in primary human cancers. *Biochem. Biophys. Res. Commun.* **111:** 47–54.

Fernandez-Capetillo, O., Chen, H.T., Celeste, A., Ward, I., Romanienko, P.J., Morales, J.C., Naka, K., Xia, Z., Camerini-Otero, R.D., Motoyama, N., et al. 2002. DNA damage-in-

duced G2-M checkpoint activation by histone H2AX and 53BP1. *Nat. Cell Biol.* **4:** 993–997.

Festenstein, R., Sharghi-Namini, S., Fox, M., Roderick, K., Tolaini, M., Norton, T., Saveliev, A., Kioussis, D., and Singh, P. 1999. Heterochromatin protein 1 modifies mammalian PEV in a dose- and chromosomal-context-dependent manner. *Nat. Genet.* **23:** 457–461.

Festenstein, R., Pagakis, S.N., Hiragami, K., Lyon, D., Verreault, A., Sekkali, B., and Kioussis, D. 2003. Modulation of heterochromatin protein 1 dynamics in primary Mammalian cells. *Science* **299:** 719–721.

Fischle, W., Wang, Y., and Allis, C.D. 2003. Binary switches and modification cassettes in histone biology and beyond. *Nature* **425:** 475–479.

Frontelo, P., Leader, J.E., Yoo, N., Potocki, A.C., Crawford, M., Kulik, M., and Lechleider, R.J. 2004. Suv39h histone methyltransferases interact with Smads and cooperate in BMP-induced repression. *Oncogene* **23:** 5242–5241.

Fuks, F., Burgers, W.A., Brehm, A., Hughes-Davies, L., and Kouzarides, T. 2000. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat. Genet.* **24:** 88–91.

Fuks, F., Hurd, P.J., Wolf, D., Nan, X., Bird, A.P., and Kouzarides, T. 2003. The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J. Biol. Chem.* **278:** 4035–4040.

Garcia-Cao, M., O'Sullivan, R., Peters, A.H., Jenuwein, T., and Blasco, M.A. 2004. Epigenetic regulation of telomere length in mammalian cells by the Suv39h1 and Suv39h2 histone methyltransferases. *Nat. Genet.* **36:** 94–99.

Gaudet, F., Hodgson, J.G., Eden, A., Jackson-Grusby, L., Dausman, J., Gray, J.W., Leonhardt, H., and Jaenisch, R. 2003. Induction of tumors in mice by genomic hypomethylation. *Science* **300:** 489–492.

Gayther, S.A., Batley, S.J., Linger, L., Bannister, A., Thorpe, K., Chin, S.F., Daigo, Y., Russell, P., Wilson, A., Sowter, H.M., et al. 2000. Mutations truncating the EP300 acetylase in human cancers. *Nat. Genet.* **24:** 300–303.

Gil, J., Bernard, D., Martinez, D., and Beach, D. 2004. Polycomb CBX7 has a unifying role in cellular lifespan. *Nat. Cell Biol.* **6:** 67–72.

Girault, I., Tozlu, S., Lidereau, R., and Bieche, I. 2003. Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. *Clin. Cancer Res.* **9:** 4415–4422.

Goelz, S.E., Vogelstein, B., Hamilton, S.R., and Feinberg, A.P. 1985. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* **228:** 187–190.

Grady, W.M., Willis, J., Guilford, P.J., Dunbier, A.K., Toro, T.T., Lynch, H., Wiesner, G., Ferguson, K., Eng, C., Park, J.G., et al. 2000. Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer. *Nat. Genet.* **26:** 16–17.

Graff, J.R., Herman, J.G., Lapidus, R.G., Chopra, H., Xu, R., Jarrard, D.F., Isaacs, W.B., Pitha, P.M., Davidson, N.E., and Baylin, S.B. 1995. E-cadherin expression is silenced by DNA hypermethylation in human breast and prostate carcinomas. *Cancer Res.* **55:** 5195–5199.

Greil, F., van der Kraan, I., Delrow, J., Smothers, J.F., de Wit, E., Bussemaker, H.J., van Driel, R., Henikoff, S., and van Steensel, B. 2003. Distinct HP1 and Su(var)3-9 complexes bind to sets of developmentally coexpressed genes depending on chromosomal location. *Genes & Dev.* **17:** 2825–2838.

Grewal, S.I. and Rice, J.C. 2004. Regulation of heterochromatin by histone methylation and small RNAs. *Curr. Opin. Cell Biol.* **16:** 230–238.

Gu, Y., Nakamura, T., Alder, H., Prasad, R., Canaani, O., Cimino, G., Croce, C.M., and Canaani, E. 1992. The t(4;11) chromosome translocation of human acute leukemias fuses the ALL-1 gene, related to *Drosophila* trithorax, to the AF-4 gene. *Cell* **71:** 701–708.

Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. 2001. A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* **27:** 322–326.

Hake, S.B., Xiao, A., and Allis, C.D. 2004. Linking the epigenetic'language' of covalent histone modifications to cancer. *Br. J. Cancer* **90:** 761–769.

Hall, I.M., Shankaranarayana, G.D., Noma, K., Ayoub, N., Cohen, A., and Grewal, S.I. 2002. Establishment and maintenance of a heterochromatin domain. *Science* **297:** 2232–2237.

Hamamoto, R., Furukawa, Y., Morita, M., Iimura, Y., Silva, F.P., Li, M., Yagyu, R., and Nakamura, Y. 2004. SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nat. Cell Biol.* **6:** 731–740.

Hanada, M., Delia, D., Aiello, A., Stadtmauer, E., and Reed, J.C. 1993. bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood* **82:** 1820–1828.

Hanahan, D. and Weinberg, R.A. 2000. The hallmarks of cancer. *Cell* **100:** 57–70.

Hansen, R.S., Wijmenga, C., Luo, P., Stanek, A.M., Canfield, T.K., Weemaes, C.M., and Gartler, S.M. 1999. The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. *Proc. Natl. Acad. Sci.* **96:** 14412–14417.

He, L., Yu, J.X., Liu, L., Buyse, I.M., Wang, M.S., Yang, Q.C., Nakagawara, A., Brodeur, G.M., Shi, Y.E., and Huang, S. 1998. RIZ1, but not the alternative RIZ2 product of the same gene, is underexpressed in breast cancer, and forced RIZ1 expression causes G2-M cell cycle arrest and/or apoptosis. *Cancer Res.* **58:** 4238–4244.

Henikoff, S., Furuyama, T., and Ahmad, K. 2004. Histone variants, nucleosome assembly and epigenetic inheritance. *Trends Genet.* **20:** 320–326.

Herman, J.G., Latif, F., Weng, Y., Lerman, M.I., Zbar, B., Liu, S., Samid, D., Duan, D.S., Gnarra, J.R., Linehan, W.M., et al. 1994. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc. Natl. Acad. Sci.* **91:** 9700–9704.

Herman, J.G., Merlo, A., Mao, L., Lapidus, R.G., Issa, J.P., Davidson, N.E., Sidransky, D., and Baylin, S.B. 1995. Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. *Cancer Res.* **55:** 4525–4530.

Herman, J.G., Umar, A., Polyak, K., Graff, J.R., Ahuja, N., Issa, J.P., Markowitz, S., Willson, J.K., Hamilton, S.R., Kinzler, K.W., et al. 1998. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc. Natl. Acad. Sci.* **95:** 6870–6875.

Hiltunen, M.O., Alhonen, L., Koistinaho, J., Myohanen, S., Paakkonen, M., Marin, S., Kosma, V.M., and Janne, J. 1997. Hypermethylation of the APC (*adenomatous polyposis coli*) gene promoter region in human colorectal carcinoma. *Int. J. Cancer* **70:** 644–648.

Huang, S. 2002. Histone methyltransferases, diet nutrients and tumour suppressors. *Nat. Rev. Cancer* **2:** 469–476.

Itahana, K., Zou, Y., Itahana, Y., Martinez, J.L., Beausejour, C., Jacobs, J.J., van Lohuizen, M., Band, V., Campisi, J., and Dimri, G.P. 2003. Control of the replicative life span of hu-

man fibroblasts by p16 and the polycomb protein Bmi-1. *Mol. Cell. Biol.* **23:** 389–401.

Ivanov, M.A., Lamrihi, B., Szyf, M., Scherman, D., and Bigey, P. 2003. Enhanced antitumor activity of a combination of MBD2-antisense electrotransfer gene therapy and bleomycin electrochemotherapy. *J. Gene Med.* **5:** 893–899.

Iyer, N.G., Ozdag, H., and Caldas, C. 2004. p300/CBP and cancer. *Oncogene* **23:** 4225–4231.

Jacobs, J.J. and van Lohuizen, M. 1999. Cellular memory of transcriptional states by Polycomb-group proteins. *Semin. Cell Dev. Biol.* **10:** 227–235.

Jacobs, J.J., Kieboom, K., Marino, S., DePinho, R.A., and van Lohuizen, M. 1999a. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* **397:** 164–168.

Jacobs, J.J., Scheijen, B., Voncken, J.W., Kieboom, K., Berns, A., and van Lohuizen, M. 1999b. Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes & Dev.* **13:** 2678–2690.

Jaenisch, R. and Bird, A. 2003. Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nat. Genet.* **(Suppl.)** **33:** 245–254.

James, S.J., Pogribny, I.P., Pogribna, M., Miller, B.J., Jernigan, S., and Melnyk, S. 2003. Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. *J. Nutr.* **133:** 3740S–3747S.

Jeddeloh, J.A., Stokes, T.L., and Richards, E.J. 1999. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. *Nat. Genet.* **22:** 94–97.

Jenuwein, T. and Allis, C.D. 2001. Translating the histone code. *Science* **293:** 1074–1080.

Jiang, G., Liu, L., Buyse, I.M., Simon, D., and Huang, S. 1999. Decreased RIZ1 expression but not RIZ2 in hepatoma and suppression of hepatoma tumorigenicity by RIZ1. *Int. J. Cancer* **83:** 541–546.

Jones, P.A. and Baylin, S.B. 2002. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* **3:** 415–428.

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:** 187–191.

Jordan, I.K., Rogozin, I.B., Glazko, G.V., and Koonin, E.V. 2003. Origin of a substantial fraction of human regulatory sequences from transposable elements. *Trends Genet.* **19:** 68–72.

Kane, M.F., Loda, M., Gaida, G.M., Lipman, J., Mishra, R., Goldman, H., Jessup, J.M., and Kolodner, R. 1997. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res.* **57:** 808–811.

Kang-Decker, N., Tong, C., Boussouar, F., Baker, D.J., Xu, W., Leontovich, A.A., Taylor, W.R., Brindle, P.K., and van Deursen, J.M. 2004. Loss of CBP causes T cell lymphomagenesis in synergy with p27Kip1 insufficiency. *Cancer Cell* **5:** 177–189.

Kanno, T., Kanno, Y., Siegel, R.M., Jang, M.K., Lenardo, M.J., and Ozato, K. 2004. Selective recognition of acetylated histones by bromodomain proteins visualized in living cells. *Mol. Cell* **13:** 33–43.

Kelly, R.G. 2003. The mouse Ink4a/Arf locus: A p53 pile-up at a tumour surveillance crossroads? *Trends Genet.* **19:** 81–83.

Khorasanizadeh, S. 2004. The nucleosome: From genomic organization to genomic regulation. *Cell* **116:** 259–272.

Kim, K.C., Geng, L., and Huang, S. 2003. Inactivation of a his-

tone methyltransferase by mutations in human cancers. *Cancer Res.* **63:** 7619–7623.

Kirschmann, D.A., Lininger, R.A., Gardner, L.M., Seftor, E.A., Odero, V.A., Ainsztein, A.M., Earnshaw, W.C., Wallrath, L.L., and Hendrix, M.J. 2000. Down-regulation of HP1Hsα expression is associated with the metastatic phenotype in breast cancer. *Cancer Res.* **60:** 3359–3363.

Kleer, C.G., Cao, Q., Varambally, S., Shen, R., Ota, I., Tomlins, S.A., Ghosh, D., Sewalt, R.G., Otte, A.P., Hayes, D.F., et al. 2003. EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc. Natl. Acad. Sci.* **100:** 11606–11611.

Klochendler-Yeivin, A., Fiette, L., Barra, J., Muchardt, C., Babinet, C., and Yaniv, M. 2000. The murine SNF5/INI1 chromatin remodeling factor is essential for embryonic development and tumor suppression. *EMBO Rep.* **1:** 500–506.

Klymenko, T. and Muller, J. 2004. The histone methyltransferases Trithorax and Ash1 prevent transcriptional silencing by Polycomb group proteins. *EMBO Rep.* **5:** 373–377.

Kung, A.L., Rebel, V.I., Bronson, R.T., Ch'ng, L.E., Sieff, C.A., Livingston, D.M., and Yao, T.P. 2000. Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP. *Genes & Dev.* **14:** 272–277.

Kuzmichev, A., Nishioka, K., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. 2002. Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. *Genes & Dev.* **16:** 2893–2905.

Kuzmichev, A., Jenuwein, T., Tempst, P., and Reinberg, D. 2004. Different ezh2-containing complexes target methylation of histone h1 or nucleosomal histone h3. *Mol. Cell* **14:** 183–193.

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:** 116–120.

Lachner, M., O'Sullivan, R.J., and Jenuwein, T. 2003. An epigenetic road map for histone lysine methylation. *J. Cell Sci.* **116:** 2117–2124.

Lagger, G., O'Carroll, D., Rembold, M., Khier, H., Tischler, J., Weitzer, G., Schuettengruber, B., Hauser, C., Brunmeir, R., Jenuwein, T., et al. 2002. Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. *EMBO J.* **21:** 2672–2681.

Lee, J.H., Park, S.J., Abraham, S.C., Seo, J.S., Nam, J.H., Choi, C., Juhng, S.W., Rashid, A., Hamilton, S.R., and Wu, T.T. 2004. Frequent CpG island methylation in precursor lesions and early gastric adenocarcinomas. *Oncogene* **23:** 4646–4654.

Lehnertz, B., Ueda, Y., Derijck, A.A., Braunschweig, U., Perez-Burgos, L., Kubicek, S., Chen, T., Li, E., Jenuwein, T., and Peters, A.H. 2003. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol.* **13:** 1192–1200.

Lessard, J. and Sauvageau, G. 2003. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* **423:** 255–260.

Leung, C., Lingbeek, M., Shakhova, O., Liu, J., Tanger, E., Saremaslani, P., van Lohuizen, M., and Marino, S. 2004. Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. *Nature* **428:** 337–341.

Lewis, A. and Murrell, A. 2004. Genomic imprinting: CTCF protects the boundaries. *Curr. Biol.* **14:** R284–R286.

Lewis, B.P., Shih, I.H., Jones-Rhoades, M.W., Bartel, D.P., and Burge, C.B. 2003. Prediction of mammalian microRNA targets. *Cell* **115:** 787–798.

Li, E. 2002. Chromatin modification and epigenetic reprogramming in mammalian development. *Nat. Rev. Genet.* **3:** 662–673.

Li, E., Bestor, T.H., and Jaenisch, R. 1992. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69:** 915–926.

Li, Y., Kirschmann, D.A., and Wallrath, L.L. 2002. Does heterochromatin protein 1 always follow code? *Proc. Natl. Acad. Sci.* (**Suppl. 4**) **99:** 16462–16469.

Lo, W.S., Trievel, R.C., Rojas, J.R., Duggan, L., Hsu, J.Y., Allis, C.D., Marmorstein, R., and Berger, S.L. 2000. Phosphorylation of serine 10 in histone H3 is functionally linked in vitro and in vivo to Gcn5-mediated acetylation at lysine 14. *Mol. Cell* **5:** 917–926.

Lund, A.H. and van Lohuizen, M. 2004. Polycomb complexes and silencing mechanisms. *Curr. Opin. Cell Biol.* **16:** 239–246.

Luo, R.X., Postigo, A.A., and Dean, D.C. 1998. Rb interacts with histone deacetylase to repress transcription. *Cell* **92:** 463–473.

Magnaghi-Jaulin, L., Groisman, R., Naguibneva, I., Robin, P., Lorain, S., Le Villain, J.P., Troalen, F., Trouche, D., and Harel-Bellan, A. 1998. Retinoblastoma protein represses transcription by recruiting a histone deacetylase. *Nature* **391:** 601–605.

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:** 329–334.

Marks, P., Rifkind, R.A., Richon, V.M., Breslow, R., Miller, T., and Kelly, W.K. 2001. Histone deacetylases and cancer: Causes and therapies. *Nat. Rev. Cancer* **1:** 194–202.

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G., and Sun, Y.E. 2003. DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* **302:** 890–893.

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:** 725–736.

Milne, T.A., Briggs, S.D., Brock, H.W., Martin, M.E., Gibbs, D., Allis, C.D., and Hess, J.L. 2002. MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol. Cell* **10:** 1107–1117.

Molofsky, A.V., Pardal, R., Iwashita, T., Park, I.K., Clarke, M.F., and Morrison, S.J. 2003. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* **425:** 962–967.

Morishita, K., Parker, D.S., Mucenski, M.L., Jenkins, N.A., Copeland, N.G., and Ihle, J.N. 1988. Retroviral activation of a novel gene encoding a zinc finger protein in IL-3-dependent myeloid leukemia cell lines. *Cell* **54:** 831–840.

Morishita, K., Parganas, E., William, C.L., Whittaker, M.H., Drabkin, H., Oval, J., Taetle, R., Valentine, M.B., and Ihle, J.N. 1992. Activation of EVI1 gene expression in human acute myelogenous leukemias by translocations spanning 300–400 kilobases on chromosome band 3q26. *Proc. Natl. Acad. Sci.* **89:** 3937–3941.

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.

Muraoka, M., Konishi, M., Kikuchi-Yanoshita, R., Tanaka, K., Shitara, N., Chong, J.M., Iwama, T., and Miyaki, M. 1996. p300 gene alterations in colorectal and gastric carcinomas. *Oncogene* **12:** 1565–1569.

Murata, T., Kurokawa, R., Krones, A., Tatsumi, K., Ishii, M., Taki, T., Masuno, M., Ohashi, H., Yanagisawa, M., Rosenfeld, M.G., et al. 2001. Defect of histone acetyltransferase activity of the nuclear transcriptional coactivator CBP in Rubinstein-Taybi syndrome. *Hum. Mol. Genet.* **10:** 1071–1076.

Myohanen, S.K., Baylin, S.B., and Herman, J.G. 1998. Hypermethylation can selectively silence individual p16ink4A alleles in neoplasia. *Cancer Res.* **58:** 591–593.

Nagai, M., Nakamura, A., Makino, R., and Mitamura, K. 2003. Expression of DNA (5-cytosin)-methyltransferases (DNMTs) in hepatocellular carcinomas. *Hepatol. Res.* **26:** 186–191.

Nakagawa, H., Chadwick, R.B., Peltomaki, P., Plass, C., Nakamura, Y., and de la Chapelle, A. 2001. Loss of imprinting of the insulin-like growth factor II gene occurs by biallelic methylation in a core region of H19-associated CTCF-binding sites in colorectal cancer. *Proc. Natl. Acad. Sci.* **98:** 591–596.

Nakagawachi, T., Soejima, H., Urano, T., Zhao, W., Higashimoto, K., Satoh, Y., Matsukura, S., Kudo, S., Kitajima, Y., Harada, H., et al. 2003. Silencing effect of CpG island hypermethylation and histone modifications on O6-methylguanine-DNA methyltransferase (MGMT) gene expression in human cancer. *Oncogene* **22:** 8835–8844.

Nakamura, M., Watanabe, T. Yonekawa, Y., Kleihues, P., and Ohgaki, H. 2001. Promoter methylation of the DNA repair gene MGMT in astrocytomas is frequently associated with G:C→A:T mutations of the TP53 tumor suppressor gene. *Carcinogenesis* **22:** 1715–1719.

Nakamura, T., Mori, T., Tada, S., Krajewski, W., Rozovskaia, T., Wassell, R., Dubois, G., Mazo, A., Croce, C.M., and Canaani, E. 2002. ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. *Mol. Cell* **10:** 1119–1128.

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:** 386–389.

Narita, M., Nunez, S., Heard, E., Narita, M., Lin, A.W., Hearn, S.A., Spector, D.L., Hannon, G.J., and Lowe, S.W. 2003. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* **113:** 703–716.

Nguyen, C.T., Gonzales, F.A., and Jones, P.A. 2001. Altered chromatin structure associated with methylation-induced gene silencing in cancer cells: Correlation of accessibility, methylation, MeCP2 binding and acetylation. *Nucleic Acids Res.* **29:** 4598–4606.

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., et al. 2001. Rb targets histone H3 methylation and HP1 to promoters. *Nature* **412:** 561–565.

Novik, K.L., Nimmrich, I., Genc, B., Maier, S., Piepenbrock, C., Olek, A., and Beck, S. 2002. Epigenomics: Genome-wide study of methylation phenomena. *Curr. Issues Mol. Biol.* **4:** 111–128.

Ogawa, H., Ishiguro, K., Gaubatz, S., Livingston, D.M., and Nakatani, Y. 2002. A complex with chromatin modifiers that occupies E2F- and Myc-responsive genes in G0 cells. *Science* **296:** 1132–1136.

Ohta, H., Sawada, A., Kim, J.Y., Tokimasa, S., Nishiguchi, S., Humphries, R.K., Hara, J., and Takihara, Y. 2002. Polycomb group gene rae28 is required for sustaining activity of hematopoietic stem cells. *J. Exp. Med.* **195:** 759–770.

Ohtani-Fujita, N., Fujita, T., Aoike, A., Osifchin, N.E., Robbins,

P.D., and Sakai, T. 1993. CpG methylation inactivates the promoter activity of the human retinoblastoma tumor-suppressor gene. *Oncogene* **8:** 1063–1067.

Okamoto, I., Otte, A.P., Allis, C.D., Reinberg, D., and Heard, E. 2004. Epigenetic dynamics of imprinted X inactivation during early mouse development. *Science* **303:** 644–649.

Okano, M., Bell, D.W., Haber, D.A., and Li, E. 1999. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* **99:** 247–257.

Orlando, V. 2003. Polycomb, epigenomes, and control of cell identity. *Cell* **112:** 599–606.

Oshimo, Y., Nakayama, H., Ito, R., Kitadai, Y., Yoshida, K., Chayama, K., and Yasui, W. 2003. Promoter methylation of cyclin D2 gene in gastric carcinoma. *Int. J. Oncol.* **23:** 1663–1670.

Oshimo, Y., Oue, N., Mitani, Y., Nakayama, H., Kitadai, Y., Yoshida, K., Chayama, K., and Yasui, W. 2004. Frequent epigenetic inactivation of RIZ1 by promoter hypermethylation in human gastric carcinoma. *Int. J. Cancer* **110:** 212–218.

Ozdag, H., Batley, S.J., Forsti, A., Iyer, N.G., Daigo, Y., Boutell, J., Arends, M.J., Ponder, B.A., Kouzarides, T., and Caldas, C. 2002. Mutation analysis of CBP and PCAF reveals rare inactivating mutations in cancer cell lines but not in primary tumours. *Br. J. Cancer* **87:** 1162–1165.

Pal, S., Yun, R., Datta, A., Lacomis, L., Erdjument-Bromage, H., Kumar, J., Tempst, P., and Sif, S. 2003. mSin3A/histone deacetylase 2- and PRMT5-containing Brg1 complex is involved in transcriptional repression of the Myc target gene cad. *Mol. Cell Biol.* **23:** 7475–7487.

Pal-Bhadra, M., Bhadra, U., and Birchler, J.A. 2002. RNAi related mechanisms affect both transcriptional and posttranscriptional transgene silencing in *Drosophila*. *Mol. Cell* **9:** 315–327.

Pal-Bhadra, M., Leibovitch, B.A., Gandhi, S.G., Rao, M., Bhadra, U., Birchler, J.A., and Elgin, S.C. 2004. Heterochromatic silencing and HP1 localization in *Drosophila* are dependent on the RNAi machinery. *Science* **303:** 669–672.

Panagopoulos, I., Fioretos, T., Isaksson, M., Samuelsson, U., Billstrom, R., Strombeck, B., Mitelman, F., and Johansson, B. 2001. Fusion of the MORF and CBP genes in acute myeloid leukemia with the t(10;16)(q22;p13). *Hum. Mol. Genet.* **10:** 395–404.

Pardal, R., Clarke, M.F., and Morrison, S.J. 2003. Applying the principles of stem-cell biology to cancer. *Nat. Rev. Cancer* **3:** 895–902.

Park, I.K., Qian, D., Kiel, M., Becker, M.W., Pihalja, M., Weissman, I.L., Morrison, S.J., and Clarke, M.F. 2003. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* **423:** 302–305.

Paz, M.F., Fraga, M.F., Avila, S., Guo, M., Pollan, M., Herman, J.G., and Esteller, M. 2003. A systematic profile of DNA methylation in human cancer cell lines. *Cancer Res.* **63:** 1114–1121.

Peters, A.H., O'Carroll, D., Scherthan, H., Mechtler, K., Sauer, S., Schofer, C., Weipoltshammer, K., Pagani, M., Lachner, M., Kohlmaier, A., et al. 2001. Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* **107:** 323–337.

Peters, A.H., Kubicek, S., Mechtler, K., O'Sullivan, R.J., Derijck, A.A., Perez-Burgos, L., Kohlmaier, A., Opravil, S., Tachibana, M., Shinkai, Y., et al. 2003. Partitioning and plasticity of repressive histone methylation states in mammalian chromatin. *Mol. Cell* **12:** 1577–1589.

Peterson, L.F. and Zhang, D.E. 2004. The 8;21 translocation in leukemogenesis. *Oncogene* **23:** 4255–4262.

Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C., Masuno, M., Tommerup, N., van Ommen, G.J., Goodman, R.H., Peters, D.J., et al. 1995. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature* **376:** 348–351.

Phelan, M.L., Sif, S., Narlikar, G.J., and Kingston, R.E. 1999. Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. *Mol. Cell* **3:** 247–253.

Plath, K., Mlynarczyk-Evans, S., Nusinow, D.A., and Panning, B. 2002. Xist RNA and the mechanism of X chromosome inactivation. *Annu. Rev. Genet.* **36:** 233–278.

Plath, K., Fang, J., Mlynarczyk-Evans, S.K., Cao, R., Worringer, K.A., Wang, H., de la Cruz, C.C., Otte, A.P., Panning, B., and Zhang, Y. 2003. Role of histone H3 lysine 27 methylation in X inactivation. *Science* **300:** 131–135.

Poetsch, M., Dittberner, T., and Woenckhaus, C. 2002. Frameshift mutations of RIZ, but no point mutations in RIZ1 exons in malignant melanomas with deletions in 1p36. *Oncogene* **21:** 3038–3042.

Pogribny, I.P., Miller, B.J., and James, S.J. 1997. Alterations in hepatic p53 gene methylation patterns during tumor progression with folate/methyl deficiency in the rat. *Cancer Lett.* **115:** 31–38.

Raaphorst, F.M., Meijer, C.J., Fieret, E., Blokzijl, T., Mommers, E., Buerger, H., Packeisen, J., Sewalt, R.A., Otte, A.P., and van Diest, P.J. 2003. Poorly differentiated breast carcinoma is associated with increased expression of the human polycomb group EZH2 gene. *Neoplasia* **5:** 481–488.

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:** 650–655.

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B.D., Sun, Z.W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C.P., Allis, C.D., et al. 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406:** 593–599.

Rego, E.M., He, L.Z., Warrell Jr., R.P., Wang, Z.G., and Pandolfi, P.P. 2000. Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML–RARα and PLZF–RARα oncoproteins. *Proc. Natl. Acad. Sci.* **97:** 10173–10178.

Reincke, B.S., Rosson, G.B., Oswald, B.W., and Wright, C.F. 2003. INI1 expression induces cell cycle arrest and markers of senescence in malignant rhabdoid tumor cells. *J. Cell. Physiol.* **194:** 303–313.

Reinhart, B.J. and Bartel, D.P. 2002. Small RNAs correspond to centromere heterochromatic repeats. *Science* **297:** 1831.

Reisman, D.N., Sciarrotta, J., Wang, W., Funkhouser, W.K., and Weissman, B.E. 2003. Loss of BRG1/BRM in human lung cancer cell lines and primary lung cancers: Correlation with poor prognosis. *Cancer Res.* **63:** 560–566.

Reyes, J.C., Barra, J., Muchardt, C., Camus, A., Babinet, C., and Yaniv, M. 1998. Altered control of cellular proliferation in the absence of mammalian brahma (SNF2α). *EMBO J.* **17:** 6979–6991.

Rice, J.C., Massey-Brown, K.S., and Futscher, B.W. 1998. Aberrant methylation of the BRCA1 CpG island promoter is associated with decreased BRCA1 mRNA in sporadic breast cancer cells. *Oncogene* **17:** 1807–1812.

Richardson, B. 2003. Impact of aging on DNA methylation. *Ageing Res. Rev.* **2:** 245–261.

Robert, M.F., Morin, S., Beaulieu, N., Gauthier, F., Chute, I.C., Barsalou, A., and MacLeod, A.R. 2003. DNMT1 is required to maintain CpG methylation and aberrant gene silencing in

human cancer cells. *Nat. Genet.* **33:** 61–65.

Roberts, C.W. and Orkin, S.H. 2004. The SWI/SNF complex—Chromatin and cancer. *Nat. Rev. Cancer* **4:** 133–142.

Roberts, C.W., Galusha, S.A., McMenamin, M.E., Fletcher, C.D., and Orkin, S.H. 2000. Haploinsufficiency of Snf5 (integrase interactor 1) predisposes to malignant rhabdoid tumors in mice. *Proc. Natl. Acad. Sci.* **97:** 13796–13800.

Roberts, C.W., Leroux, M.M., Fleming, M.D., and Orkin, S.H. 2002. Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. *Cancer Cell* **2:** 415–425.

Robertson, K.D., Uzvolgyi, E., Liang, G., Talmadge, C., Sumegi, J., Gonzales, F.A., and Jones, P.A. 1999. The human DNA methyltransferases (DNMTs) 1, 3a and 3b: Coordinate mRNA expression in normal tissues and overexpression in tumors. *Nucleic Acids Res.* **27:** 2291–2298.

Robertson, K.D., Ait-Si-Ali, S., Yokochi, T., Wade, P.A., Jones, P.L., and Wolffe, A.P. 2000. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat. Genet.* **25:** 338–342.

Rougeulle, C. and Heard, E. 2002. Antisense RNA in imprinting: Spreading silence through air. *Trends Genet.* **18:** 434–437.

Rountree, M.R., Bachman, K.E., and Baylin, S.B. 2000. DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat. Genet.* **25:** 269–277.

Rozenblatt-Rosen, O., Rozovskaia, T., Burakov, D., Sedkov, Y., Tillib, S., Blechman, J., Nakamura, T., Croce, C.M., Mazo, A., and Canaani, E. 1998. The C-terminal SET domains of ALL-1 and TRITHORAX interact with the INI1 and SNR1 proteins, components of the SWI/SNF complex. *Proc. Natl. Acad. Sci.* **95:** 4152–4157.

Sansom, O.J., Berger, J., Bishop, S.M., Hendrich, B., Bird, A., and Clarke, A.R. 2003. Deficiency of Mbd2 suppresses intestinal tumorigenesis. *Nat. Genet.* **34:** 145–147.

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:** 407–411.

Sasaki, O., Meguro, K., Tohmiya, Y., Funato, T., Shibahara, S., and Sasaki, T. 2002. Nucleotide alteration of retinoblastoma protein-interacting zinc finger gene, RIZ, in human leukemia. *Tohoku J. Exp. Med.* **196:** 193–201.

Schmiedeberg, L., Weisshart, K., Diekmann, S., Meyer Zu, H.G., and Hemmerich, P. 2004. High and low mobility populations of HP1 in heterochromatin of mammalian cells. *Mol. Biol. Cell.* **15:** 2819–2833.

Schneider, R., Bannister, A.J., and Kouzarides, T. 2002. Unsafe SETs: Histone lysine methyltransferases and cancer. *Trends Biochem. Sci.* **27:** 396–402.

Schramke, V. and Allshire, R. 2003. Hairpin RNAs and retrotransposon LTRs effect RNAi and chromatin-based gene silencing. *Science* **301:** 1069–1074.

Schulz, W.A., Elo, J.P., Florl, A.R., Pennanen, S., Santourlidis, S., Engers, R., Buchardt, M., Seifert, H.H., and Visakorpi, T. 2002. Genomewide DNA hypomethylation is associated with alterations on chromosome 8 in prostate carcinoma. *Genes Chromosomes Cancer* **35:** 58–65.

Sevenet, N., Sheridan, E., Amram, D., Schneider, P., Handgretinger, R., and Delattre, O. 1999. Constitutional mutations of the hSNF5/INI1 gene predispose to a variety of cancers. *Am. J. Hum. Genet.* **65:** 1342–1348.

Sewalt, R.G., Lachner, M., Vargas, M., Hamer, K.M., den Blaauwen, J.L., Hendrix, T., Melcher, M., Schweizer, D., Jenuwein, T., and Otte, A.P. 2002. Selective interactions between vertebrate polycomb homologs and the SUV39H1 histone lysine methyltransferase suggest that histone H3-K9 methylation contributes to chromosomal targeting of Polycomb group proteins. *Mol. Cell. Biol.* **22:** 5539–5553.

Shi, Y., Sawada, J., Sui, G., Affar, el B., Whetstine, J.R., Lan, F., Ogawa, H., Luke, M.P., Nakatani, Y., and Shi, Y. 2003. Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* **422:** 735–738.

Silva, J., Mak, W., Zvetkova, I., Appanah, R., Nesterova, T.B., Webster, Z., Peters, A.H., Jenuwein, T., Otte, A.P., and Brockdorff, N. 2003. Establishment of histone h3 methylation on the inactive X chromosome requires transient recruitment of Eed–Enx1 polycomb group complexes. *Dev. Cell* **4:** 481–495.

Sitailo, S., Sood, R., Barton, K., and Nucifora, G. 1999. Forced expression of the leukemia-associated gene EVI1 in ES cells: A model for myeloid leukemia with 3q26 rearrangements. *Leukemia* **13:** 1639–1645.

Sleutels, F., Zwart, R., and Barlow, D.P. 2002. The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* **415:** 810–813.

So, C.W. and Cleary, M.L. 2004. Dimerization: A versatile switch for oncogenesis. *Blood.* **104:** 919–922.

So, C.W., Lin, M., Ayton, P.M., Chen, E.H., and Cleary, M.L. 2003. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. *Cancer Cell* **4:** 99–110.

Sobulo, O.M., Borrow, J., Tomek, R., Reshmi, S., Harden, A., Schlegelberger, B., Housman, D., Doggett, N.A., Rowley, J.D., and Zeleznik, L. 1997. MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). *Proc. Natl. Acad. Sci.* **94:** 8732–8737.

Soderholm, J., Kobayashi, H., Mathieu, C., Rowley, J.D., and Nucifora, G. 1997. The leukemia-associated gene MDS1/EVI1 is a new type of GATA-binding transactivator. *Leukemia* **11:** 352–358.

Soejima, K., Fang, W., and Rollins, B.J. 2003. DNA methyltransferase 3b contributes to oncogenic transformation induced by SV40T antigen and activated Ras. *Oncogene* **22:** 4723–4733.

Steele-Perkins, G., Fang, W., Yang, X.H., Van Gele, M., Carling, T., Gu, J., Buyse, I.M., Fletcher, J.A., Liu, J., Bronson, R., et al. 2001. Tumor formation and inactivation of RIZ1, an Rb-binding member of a nuclear protein-methyltransferase superfamily. *Genes & Dev.* **15:** 2250–2262.

Strahl, B.D. and Allis, C.D. 2000. The language of covalent histone modifications. *Nature* **403:** 41–45.

Sudarsanam, P., Iyer, V.R., Brown, P.O., and Winston, F. 2000. Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **97:** 3364–3369.

Sun, L.Q., Lee, D.W., Zhang, Q., Xiao, W., Raabe, E.H., Meeker, A., Miao, D., Huso, D.L., and Arceci, R.J. 2004. Growth retardation and premature aging phenotypes in mice with disruption of the SNF2-like gene, PASG. *Genes & Dev.* **18:** 1035–1046.

Sung, S. and Amasino, R.M. 2004. Vernalization and epigenetics: How plants remember winter. *Curr. Opin. Plant Biol.* **7:** 4–10.

Suter, C.M., Martin, D.I., and Ward, R.L. 2004. Germline epimutation of MLH1 in individuals with multiple cancers. *Nat. Genet.* **36:** 497–501.

Tagami, H., Ray-Gallet, D., Almouzni, G., and Nakatani, Y. 2004. Histone h3.1 and h3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* **116:** 51–61.

Tam, W., Ben Yehuda, D., and Hayward, W.S. 1997. bic, a novel gene activated by proviral insertions in avian leukosis virus-induced lymphomas, is likely to function through its non-coding RNA. *Mol. Cell. Biol.* **17:** 1490–1502.

Tam, W., Hughes, S.H., Hayward, W.S., and Besmer, P. 2002. Avian bic, a gene isolated from a common retroviral site in avian leukosis virus-induced lymphomas that encodes a noncoding RNA, cooperates with c-myc in lymphomagenesis and erythroleukemogenesis. *J. Virol.* **76:** 4275–4286.

Tamaru, H. and Selker, E.U. 2001. A histone H3 methyltransferase controls DNA methylation in Neurospora crassa. *Nature* **414:** 277–283.

Tamkun, J.W., Deuring, R., Scott, M.P., Kissinger, M., Pattatucci, A.M., Kaufman, T.C., and Kennison, J.A. 1992. brahma: A regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68:** 561–572.

Taylor, M.D., Gokgoz, N., Andrulis, I.L., Mainprize, T.G., Drake, J.M., and Rutka, J.T. 2000. Familial posterior fossa brain tumors of infancy secondary to germline mutation of the hSNF5 gene. *Am. J. Hum. Genet.* **66:** 1403–1406.

Tkachuk, D.C., Kohler, S., and Cleary, M.L. 1992. Involvement of a homolog of *Drosophila* trithorax by 11q23 chromosomal translocations in acute leukemias. *Cell* **71:** 691–700.

Tomonaga, T., Matsushita, K., Yamaguchi, S., Oohashi, T., Shimada, H., Ochiai, T., Yoda, K., and Nomura, F. 2003. Overexpression and mistargeting of centromere protein-A in human primary colorectal cancer. *Cancer Res.* **63:** 3511–3516.

Toyota, M., Ahuja, N., Ohe-Toyota, M., Herman, J.G., Baylin, S.B., and Issa, J.P. 1999a. CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci.* **96:** 8681–8686.

Toyota, M., Ahuja, N., Suzuki, H., Itoh, F., Ohe-Toyota, M., Imai, K., Baylin, S.B., and Issa, J.P. 1999b. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res.* **59:** 5438–5442.

Tsuda, H., Takarabe, T., Kanai, Y., Fukutomi, T., and Hirohashi, S. 2002. Correlation of DNA hypomethylation at pericentromeric heterochromatin regions of chromosomes 16 and 1 with histological features and chromosomal abnormalities of human breast carcinomas. *Am. J. Pathol.* **161:** 859–866.

Tufarelli, C., Stanley, J.A., Garrick, D., Sharpe, J.A., Ayyub, H., Wood, W.G., and Higgs, D.R. 2003. Transcription of antisense RNA leading to gene silencing and methylation as a novel cause of human genetic disease. *Nat. Genet.* **34:** 157–165.

Valk-Lingbeek, M.E., Bruggeman, S.W.M., and van Lohuizen, M. 2004. Stem cells and cancer: The Polycomb connection. *Cell* **118:** 409–418.

van den Berg, A., Kroesen, B.J., Kooistra, K., de Jong, D., Briggs, J., Blokzijl, T., Jacobs, S., Kluiver, J., Diepstra, A., Maggio, E., et al. 2003. High expression of B-cell receptor inducible gene BIC in all subtypes of Hodgkin lymphoma. *Genes Chromosomes Cancer* **37:** 20–28.

van der Vlag, J. and Otte, A.P. 1999. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. *Nat. Genet.* **23:** 474–478.

van Lohuizen, M., Verbeek, S., Scheijen, B., Wientjens, E., van der Gulden, H., and Berns, A. 1991. Identification of cooperating oncogenes in E μ-myc transgenic mice by provirus tagging. *Cell* **65:** 737–752.

Vandel, L., Nicolas, E., Vaute, O., Ferreira, R., Ait-Si-Ali, S., and Trouche, D. 2001. Transcriptional repression by the retino-

blastoma protein through the recruitment of a histone methyltransferase. *Mol. Cell. Biol.* **21:** 6484–6494.

Varambally, S., Dhanasekaran, S.M., Zhou, M., Barrette, T.R., Kumar-Sinha, C., Sanda, M.G., Ghosh, D., Pienta, K.J., Sewalt, R.G., Otte, A.P., et al. 2002. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* **419:** 624–629.

Vaute, O., Nicolas, E., Vandel, L., and Trouche, D. 2002. Functional and physical interaction between the histone methyl transferase Suv39H1 and histone deacetylases. *Nucleic Acids Res.* **30:** 475–481.

Velicescu, M., Weisenberger, D.J., Gonzales, F.A., Tsai, Y.C., Nguyen, C.T., and Jones, P.A. 2002. Cell division is required for de novo methylation of CpG islands in bladder cancer cells. *Cancer Res.* **62:** 2378–2384.

Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M.F., Ambros, P., Handgretinger, R., Aurias, A., and Delattre, O. 1998. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature* **394:** 203–206.

Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I., and Martienssen, R.A. 2002. Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* **297:** 1833–1837.

Vonlanthen, S., Heighway, J., Altermatt, H.J., Gugger, M., Kappeler, A., Borner, M.M., van Lohuizen, M., and Betticher, D.C. 2001. The bmi-1 oncoprotein is differentially expressed in non-small cell lung cancer and correlates with INK4A-ARF locus expression. *Br. J. Cancer* **84:** 1372–1376.

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:** 62–66.

Waki, T., Tamura, G., Sato, M., Terashima, M., Nishizuka, S., and Motoyama, T. 2003. Promoter methylation status of DAP-kinase and RUNX3 genes in neoplastic and non-neoplastic gastric epithelia. *Cancer Sci.* **94:** 360–364.

Wang, H., Huang, Z.Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B.D., Briggs, S.D., Allis, C.D., Wong, J., Tempst, P., et al. 2001a. Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science* **293:** 853–857.

Wang, J., Mager, J., Chen, Y., Schneider, E., Cross, J.C., Nagy, A., and Magnuson, T. 2001b. Imprinted X inactivation maintained by a mouse Polycomb group gene. *Nat. Genet.* **28:** 371–375.

Weaver, V.M. and Gilbert, P. 2004. Watch thy neighbor: Cancer is a communal affair. *J. Cell Sci.* **117:** 1287–1290.

Weinmann, A.S., Bartley, S.M., Zhang, T., Zhang, M.Q., and Farnham, P.J. 2001. Use of chromatin immunoprecipitation to clone novel E2F target promoters. *Mol. Cell. Biol.* **21:** 6820–6832.

Whang, Y.E., Wu, X., Suzuki, H., Reiter, R.E., Tran, C., Vessella, R.L., Said, J.W., Isaacs, W.B., and Sawyers, C.L. 1998. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. *Proc. Natl. Acad. Sci.* **95:** 5246–5250.

Wong, A.K., Shanahan, F., Chen, Y., Lian, L., Ha, P., Hendricks, K., Ghaffari, S., Iliev, D., Penn, B., Woodland, A.M., et al. 2000. BRG1, a component of the SWI–SNF complex, is mutated in multiple human tumor cell lines. *Cancer Res.* **60:** 6171–6177.

Xu, G.L., Bestor, T.H., Bourc'his, D., Hsieh, C.L., Tommerup, N., Bugge, M., Hulten, M., Qu, X., Russo, J.J., and Viegas-Pequignot, E. 1999. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. *Nature* **402:** 187–191.

Xu, W., Chen, H., Du, K., Asahara, H., Tini, M., Emerson, B.M., Montminy, M., and Evans, R.M. 2001. A transcriptional switch mediated by cofactor methylation. *Science* **294:** 2507–2511.

Yan, Q., Huang, J., Fan, T., Zhu, H., and Muegge, K. 2003. Lsh, a modulator of CpG methylation, is crucial for normal histone methylation. *EMBO J.* **22:** 5154–5162.

Yang, X.J. 2004. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. *Nucleic Acids Res.* **32:** 959–976.

Yao, T.P., Oh, S.P., Fuchs, M., Zhou, N.D., Ch'ng, L.E., Newsome, D., Bronson, R.T., Li, E., Livingston, D.M., and Eckner, R. 1998. Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300. *Cell* **93:** 361–372.

Yoda, K., Ando, S., Morishita, S., Houmura, K., Hashimoto, K., Takeyasu, K., and Okazaki, T. 2000. Human centromere protein A (CENP-A) can replace histone H3 in nucleosome reconstitution in vitro. *Proc. Natl. Acad. Sci.* **97:** 7266–7271.

Yoder, J.A., Walsh, C.P., and Bestor, T.H. 1997. Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet.* **13:** 335–340.

Zeng, L. and Zhou, M.M. 2002. Bromodomain: An acetyl-lysine binding domain. *FEBS Lett.* **513:** 124–128.

Zhu, P., Martin, E., Mengwasser, J., Schlag, P., Janssen, K.P., and Gottlicher, M. 2004a. Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. *Cancer Cell* **5:** 455–463.

Zhu, Y., Spitz, M.R., Zhang, H., Grossman, H.B., Frazier, M.L., and Wu, X. 2004b. Methyl-CpG-binding domain 2: A protective role in bladder carcinoma. *Cancer* **100:** 1853–1858.

Epigenetics and cancer

Anders H. Lund and Maarten van Lohuizen

References	This article cites 280 articles, 102 of which can be accessed free at: **http://genesdev.cshlp.org/content/18/19/2315.full.html#ref-list-1**
License	
Email Alerting Service	Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or **click here.**