Talk is cheap––cross-talk in establishment, maintenance, and readout of chromatin modifications

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PERSPECTIVE

Talk is cheap—cross-talk in establishment, maintenance, and readout of chromatin modifications

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The functionality of a cell's genome is controlled epigenetically on the level of chromatin. Multiple post-translational modifications of histone proteins together with DNA methylation play a key role in directing distinct functional states of chromatin. As it emerges, many epigenetic marks on the chromatin platform do not act independently, but cross-talk with each other. In this issue of *Genes* & *Development*, Adhvaryu and Selker (pp. 3391–3396) provide novel insights into an intricate regulatory network involving histone phosphorylation, histone methylation, and DNA methylation.

The physiological template of genetic information in all eukaryotic cells is chromatin, a nucleo–protein complex composed mainly of DNA and histone proteins plus the addition of other proteins as well as RNAs. As a biological relay station and signaling platform, chromatin integrates a variety of endogenous and exogenous cellular inputs. The various signals are thought to direct distinct local and global functional states of chromatin, therefore controlling the capacity of a cell's genome to store, release, and inherit biological information. Commonly, the sum of such processes is referred to as epigenetic, albeit in their strictest definition only inheritable changes in gene expression without variation in DNA sequence qualify as epigenetic. Epigenetic variation is now considered a key component of many diseases, including cancer.

In the repeating unit of chromatin, the nucleosome, DNA is wrapped around an octamer of core histone proteins (two copies each of H2A, H2B, H3, and H4), an arrangement that is further stabilized by linker histones of the H1 type. The architecture of chromatin on the nucleosomal level is basically identical for all of the genome. Besides incorporation of histone variants, divergence is achieved via regional restricted methylation of DNA as well as numerous post-translational modifications (PTM) of the histone proteins (chromatin marks). Histones can be modified in a number of ways including acetylation (ac), monomethylation (me1), dimethylation (me2), trimethylation (me3), and ubiquitination (ub) of lysine residues; monomethylation (me1), symmetrical dimethylation (symme2), and asymmetrical dimethylation (asymme2) of arginine residues as well as phosphorylation of serine and threonine residues (ph). Combinations of chromatin marks regulate chromatin structure, thereby defining different functional domains of chromatin. The most recognized domains of chromatin are euchromatin (commonly referred to as the "active" form of chromatin) and heterochromatin (commonly referred to as the "inactive" form of chromatin). New work by Adhvaryu and Selker (2008) implicates histone methylation, histone phosphorylation, and DNA methylation in a chromatin signaling network for silencing of defined regions of the genome.

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DNA methylation

Methylation of cytosine bases (meC) in DNA occurs to varying degrees in a wide range of organisms, from fungi and plants to mammals. Whereas DNA methylation in mammals is restricted to symmetric CpG sites, plants in addition contain methylated cytosines in CpNpG and CpHpH environments (N any base; H not G). In filamentous fungi like *Neurospora crassa* a genome defense mechanisms against mobile elements called RIP (repeat-induced point mutation) also results in meC in a non-symmetric sequence context.

DNA methylation generally plays a role in silencing gene expression in both heterochromatin and euchromatin domains. Especially in mammals it is implicated in genomic imprinting and X inactivation, and its loss leads to growth arrest or apoptosis. Most DNA methylation patterns are established in defined windows of differentiation and development, but changes due to environmental stimuli or in pathologic responses such as tumorigenesis occur. In mammals and plants genomic DNA methylation imprints are established very early during embryonic development. In *Neurospora* RIP only takes place during the sexual cycle of the organism. De novo DNA methyltransferases (DNMT) like the mam-

malian DNMT3a and DNMT3b proteins that act together with the nonenzymatic DNMT3L are thought to establish the methylation patterns. Since DNA methylation plays such an important role in genome regulation, its targeting mechanisms are of great interest (discussed recently by Woo and Richards 2008). While de novo DNMTs might have some weak intrinsic site specificity, association with sequence-specific binding proteins and targeting by small RNAs seem to be involved in this process. Further, cross-talk with histone modifications has emerged as pivotal in defining and establishing DNA methylation. Different targets of DNA methylation are (1) parentally imprinted genes as key regulators of embryonic development and adult life; (2) intergenic DNA, exons, proviral genomes, and retrotransposons, as well as other repeated sequences; and (3) a number of genes that are methylated in a tissue-specific manner.

Existing patterns of DNA methylation are faithfully copied by semiconservative maintenance DNMTs. These enzymes replicate a given methylation pattern by methylating a hemimethylated template originating from DNA replication. The family of maintenance DNMTs contains the paradigm mammalian DNMT1, the plant MET1, which maintains CpG methylation, as well as plant CMT3 and DRM2 factors, which sustain non-CpG methylation. *Neurospora* contains a single DNMT, DIM-2, that is implicated in de novo and maintenance methylation (Vaillant and Paszkowski 2007). While the templating mechanisms of DNA methylation patterns seemingly allow stable epigenetic marking of the genome, it is becoming evident that this system interfaces tightly with histone modifications for efficient and faithful transmission through multiple cell divisions.

The methyl moiety of methyl-cytosine resides in the major groove of the DNA double helix. There, many DNA-binding proteins make contact with DNA. Three families of proteins have been shown capable of recognizing methylated CpG: (1) members of the methyl-CpG-binding domain (MBD) family (like the mammalian MeCP2, and MBD1-4); (2) a family of Zinc-finger proteins, which bind methylated DNA, but also some non-methylated consensus sequences, containing Kaiso, ZBT4, and ZBTB38; and (3) factors containing SRA (SET- and RING-finger-associated) domains like UHRF1/ICBP90. Other transcription factors bind to CpG-containing DNA sequences only when these are nonmethylated. Since methylated DNA when introduced into mammalian cells or frog oocytes initially permits gene transcription but is silenced after several hours, it was suggested early on that readout of the meC mark is indirect and requires assembly of chromatin. Indeed, several meC-binding factors have been shown to recruit histone-modifying repressor complexes for transcriptional silencing.

Histone methylation—H3K9me

Besides DNA methylation, histone PTMs constitute the second major covalent regulatory system of chromatin. Many different sites of histone modification on the core and linker histones have been identified to date that alone or in combination could control distinct chromatin states and that appear to be part of epigenetic regulatory systems. Methylation of several lysine residues in histones H3 and H4 has attracted a lot of attention as these chromatin marks have been implicated in diverse biological processes from transcriptional activation and elongation to gene repression and from DNA repair to DNA replication depending on the site and status (me1, me2, or me3) of the different targeted histone lysine residues.

Methylation of Lys 9 of histone H3 (H3K9me) has been mainly correlated to gene silencing. Different subnuclear distribution of the me1, me2, and me3 forms of this chromatin mark indicate potential distinct functions. For example, H3K9me3 has been mapped to heterochromatin in different experimental systems from yeast to man. Plants are an exception. There, H3K9me3 maps to euchromatin, but H3K9me2 is instead enriched at heterochromatic sites (Vaillant and Paszkowski 2007). Since heterologous targeting of histone lysine methyltransferases (KMT) mediating H3K9me3 results in induction of gene silencing accompanied by chromatin condensation (Schulze and Wallrath 2007), this modification is widely considered a repressive chromatin mark. Nevertheless, H3K9me3 was also found distributed to the body of active genes (Vakoc et al. 2005).

Different enzymes have been shown to target H3K9 for methylation. As for other sites of histone methylation, these display not only specificity for the H3K9 residue, but also mediate very distinct levels of methylation of this site. The founding member of the H3K9MTs is Su(var)3-9, which was first characterized as a suppressor of variegation in *Drosophila*. In mammals, the two Suv39h1/h2 isoforms seem to primarily mediate H3K9me2 and H3K9me3 in pericentromeric heterochromatin, while mono- and dimethylation of H3K9 in euchromatic regions are catalyzed by the G9a/GLP complex. In contrast, SETDB1 might mediate all states of H3K9 methylation. Plants contain a family of 10 putative H3K9MTs (SUV1–10), all possibly involved in distinct and overlapping functions in the establishment and maintenance of this epigenetic mark. In *Neurospora*, DIM-5 is the paradigm H3K9MT, mediating all states of methylation of this site (Volkel and Angrand 2007).

Similar to DNA methylation, mechanisms for targeting and establishing H3K9me include interaction of KMTs with sequence-specific DNA-binding proteins as well as pathways guided by small RNAs (Grewal and Elgin 2007). In several instances, cross-talk with DNA methylation seems to play an important role in directing patterns of this chromatin mark.

The biological systems that ensure faithful transmission of H3K9me from one cell generation to the next are still unclear. Recent evidence of replication-dependent deposition of H3–H4 dimers instead of preformed $(H3–H4)_2$ tetramers have given rise to the idea that histone modification marks might also be inherited in a semiconservative fashion from mother to daughter chromatid

(Nakatani et al. 2004). Since several enzymes for demethylation of H3K9me have been described, the once thought biological stability and epigenetic indexing potential of this chromatin mark is in question. While the systems for establishing H3K9me at heterochromatin appear to be constantly in play in lower eukaryotes such as *Schizosaccharomyces pombe,* tight interplay with DNA methylation might provide reinforcement for this chromatin mark in higher eukaryotes.

A number of proteins have been shown to specifically interact with H3K9me. HP1 binds via a so-called chromodomain to histone H3 peptides in vitro with a slight preference for K9me2/3 over K9me1. Largely overlapping cellular distribution of HP1 with H3K9me3 is in agreement with a role of this factor in mediating H3K9me3 function. Ankyrin repeats of G9a were more recently shown to bind H3K9me1/2 in vitro (Collins et al. 2008). Also, a PHD region in UHRF1 displays affinity for H3K9me (Papait et al. 2008). However, the downstream working mechanisms of the different H3K9me interacting factors are still unknown. An important aspect of H3K9me biology seems to be cross-talk with DNA methylation systems.

Histone phosphorylation—H3S10ph

Phosphorylation of several serine and threonine residues in the core and linker histones has been implicated in gene regulation as well as in cell cycle-dependent chromosome condensation, DNA repair, and apoptosis-induced chromatin compaction. Phosphorylation of H3S10 during mitosis and meiosis seems to be required for proper chromosome segregation and cell cycle progression. In response to stress or mitogen-stimulated signaling pathways H3S10ph was implicated in transcriptional activation in mammals and plants (Houben et al. 2007). Also, H3S10ph facilitates Pol II clearance from promoter-proximal pausing in *Drosophila* (Ivaldi et al. 2007). However, H3S10ph has also been connected to silent chromatin in post-mitotic mammalian cells (Sabbattini et al. 2007), and it was correlated to heterochromatin in interphase cells of plants (Houben et al. 2007).

H3S10 is phosphorylated by multiple kinases, such as MSK1, MSK2, PKA, RSK2, and IKKα. These kinases are locally recruited to promoter regions of target genes by interaction with sequence-specific DNA-binding proteins. Mitotic kinases, NIMA and Aurora B, in contrast, target H3S10 for global, chromosomal phosphorylation. Whereas NIMA in filamentous fungi seems to be localized to the spindle pole body, the major microtubule organizing center and functional equivalent of the higher eukaryotic centrosome, Aurora B, is the enzymatic component of the so-called chromosomal passenger complex. During the progress of mitosis, this complex is sequentially found nuclear-, centromeric-, and then mid-spindle- and mid-body-associated. Another kinase with seemingly global function is the JIL-1 enzyme of *Drosophila* that is generally found in regions of euchromatin where it is thought to counteract heterochromatinization.

As with phosphorylation in other signaling systems, H3S10ph is very transient and requires permanent presence of modifying kinases. Kinase enzymatic inhibitors shift the balance toward the dephosphorylated state due to the obviously continuous action of phosphatases. Whereas the analysis of H3S10ph dephosphorylation lags behind the study of kinases, PP1 has been implicated in removing the phospho-mark in different experimental systems. For example, the PP1 enzyme Glc7 counteracts Aurora B H3S10 phosphorylation in *Saccharomyces cerevisiae* and *Caenorhabditis elegans.*

The enigma of correlation of H3S10ph with two apparently opposed chromatin states (transcriptionally active decondensed euchromatin versus condensed mitotic chromosomes and silent heterochromatin) is not clearly understood. Since H3S10ph by itself has no direct effect on higher-order chromatin folding (Shogren-Knaak et al. 2003), genomic and chromatin context likely plays an important role in its biology. For example, the acetylation state of H3K9 and H3K14 influences binding of 14–3–3 proteins to H3S10ph, and thereby readout of this chromatin mark in mitogen-stimulated signaling (Macdonald et al. 2005; Winter et al. 2008). Interestingly, earlier studies showed that H3S10ph could enhance acetylation of the neighboring K14 residue and abolish acetylation of the adjacent K9 site (Fischle et al. 2003). Further evidence for indirect readout of H3S10ph comes from studies implicating H3K9me in its function.

Cross-talk between chromatin marks

The high density of sites and various types of histone PTMs plus additional DNA methylation might indicate that many chromatin marks like H3S10ph are not acting independently, but influence each other function. Such cross-talk can come in multiple flavors and is manifested by various mechanisms and on distinct chromatin levels (Fig. 1). The first indication for cross-talk of chromatin marks came by biochemical analysis of the substrate specificity of histone-modifying enzymes (for review, see Fischle et al. 2003). There, pre-existing PTMs can enhance or decrease subsequent additional modification. Mechanistically, these effects are manifested on the level of enzyme recruitment as well as substrate recognition and/or turnover. The simplest case is obviously the blocking of modification of a site by another pre-existing mark (e.g., H3K9ac blocking H3K9 methylation). More intriguing are cases where an enzyme is apparently activated by interaction via a chromatin mark for modification of another site such as in the case of the Dot1 KMT, which is stimulated by ubiquitylated histone H2B for intranucleosomal lysine methylation (McGinty et al. 2008). Next to direct cross-talk of chromatin marks in the establishment of modification patterns via influence on enzymes, indirect cross-talk on the level of factors binding to chromatin marks has been observed, thereby establishing synergistic or antagonistic readout of multiple modifications (Taverna et al. 2007).

While in vitro data for different cross-talk scenarios are very clear, in vivo evidence for such interplay of chro-

matin marks is more difficult to obtain. Extremely powerful are experiments where mutagenesis of sites of histone modification is undertaken. Unfortunately, such approaches are limited to lower eukaryotes, which have only a few histone genes, as most higher eukaryotes contain multiple gene loci encoding for the different histone proteins. The histone mutagenesis studies are further complicated by the fact that single sites of modification can be targets of multiple cross-talk. For example, a given modification might cross-talk with another chromatin mark in an indirect readout, but be also subject to direct cross-talk for modification/demodification (i.e., modification of a site is blocked by mutagenesis of another site as the modifying enzyme does not recognize the mutated substrate). The technical and intrinsic restrictions of histone mutagenesis studies leaves, in many cases, approaches where chromatin-modifying enzymes are mutated as the next best choice. Both types of experiments are nicely combined by Adhvaryu and Selker (2008). Also, a combination of biochemical in vitro studies with correlative analysis of cellular scenarios has proven useful in the study of cross-talk of chromatin marks. A system where extensive cross-talk is emerging is the H3K9me, H3S10ph, and DNA methylation network.

Cross-talk between H3K9me and H3S10ph

Early indication for a negative, directly inhibiting cross-talk between H3S10ph and H3K9me on the same histone tail came from the analysis of the enzymatic activity of the H3K9KMT Suvar39, which showed reduced methylation on an H3S10ph peptide compared with the unmodified substrate (see Fig. 1B; Rea et al. 2000).

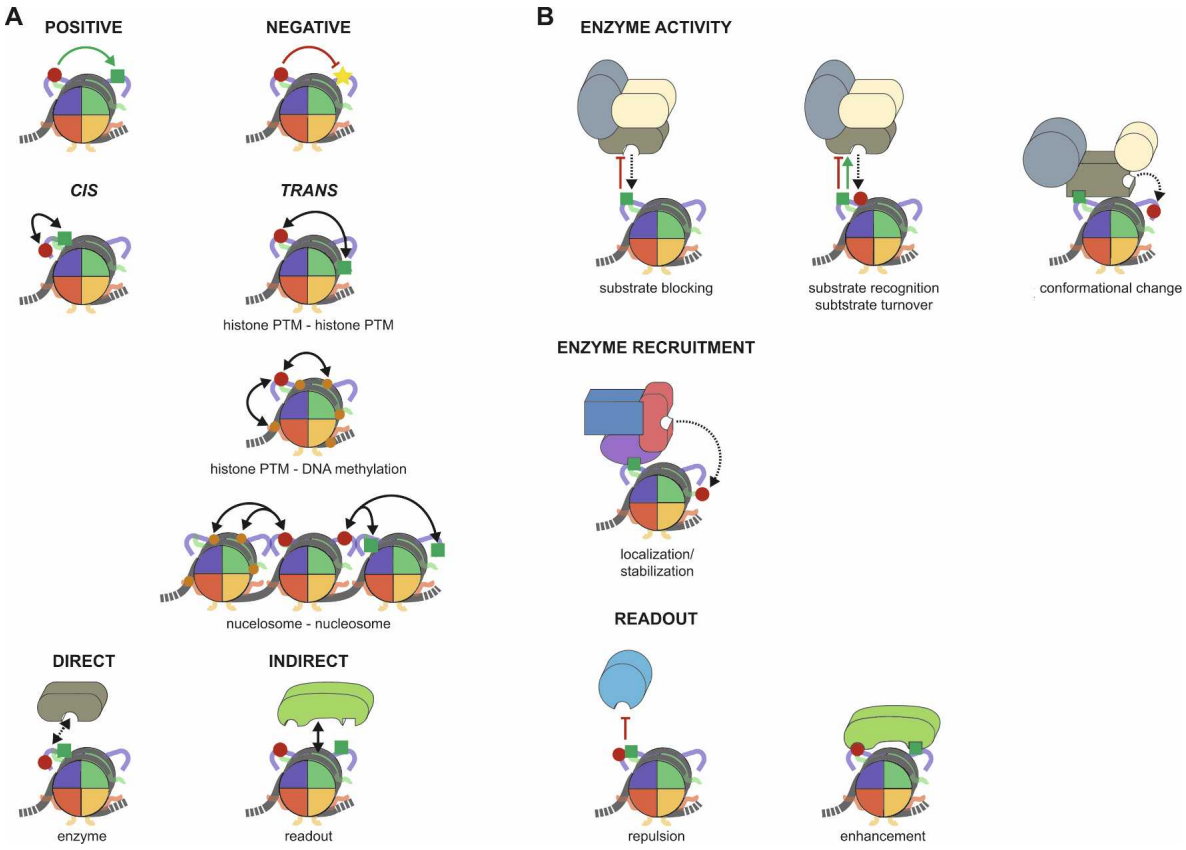

Figure 1. Modes and mechanisms of chromatin modification cross-talk. (*A*) Chromatin modifications can enhance (positive cross-talk) or block (negative cross-talk) each other's function. Cross-talk can be restricted to a single histone molecule within the nucleosome (*cis* effect). Alternatively, cross-talk can occur between histone PTMs on different histones, between different histone PTMs and DNA methylation, or between chromatin modifications on adjacent nucelosomes (*trans* effect). Cross-talk is possible directly on the level of modifying enzymes or indirectly through binding/readout factors. (*B*) Existing modifications can directly block enzymatic activity directed toward the same site/residue. Also, certain modification states (unmodified vs. modified) represent better or worse substrates for chromatin-modifying enzymes. Binding to a pre-existing modification can activate an enzyme/enzyme complex. Enzymatic activity might also be localized and stabilized on target regions via binding to pre-existing chromatin marks. Further, in indirect cross-talk, additional modifications might enhance or block the binding of a readout factor. Nucleosomes are depicted schematically, with DNA (gray) wrapped around a disk of core histones H2A (yellow), H2B (red), H3 (blue), and H4 (green); core histone N-terminal tails are schematized. Red dots, green boxes, and yellow stars represent different histone PTMs; orange dots represent DNA methylation. (Green) Positive cross-talk; (red) negative cross-talk; (black) positive or negative cross-talk; (dashed lines) enzymatic activities.

Genetic evidence for cross-talk between H3S10ph and H3K9me is derived largely from the study of JIL-1 kinase in *Drosophila*. It could be shown that loss-of-function mutation of JIL-1 or reduction in its expression levels causes spreading of major heterochromatin markers, H3K9me and HP1 binding (Zhang et al. 2006; Bao et al. 2007). Conversely, artificial recruitment of JIL-1 to a condensed heterochromatinized gene array results in chromatin structure remodeling toward a more open euchromatic state (Deng et al. 2008). Unbiased genetic analysis further identified gain-of-function mutations in JIL-1 as Su(var)3-1 alleles strongly counteracting the repressive effect of Su(var)3-9 (Ebert et al. 2004). Since these studies detected no impairment on the distribution and levels of H3K9me, a plausible scenario for a molecular mechanism of the antagonizing effects of H3S10ph onto H3K9me3 and heterochromatin is that H3S10ph prevents the binding and/or activity of condensing factors, thereby inducing an euchromatic state (see Fig. 1A,B).

Support for indirect effects of H3S10ph onto the readout of H3K9me3 came first from the analysis of HP1 behavior in mitosis of mammalian cells. During M-phase, a large portion of the heterochromatin-localized HP1 is displaced from chromatin despite unchanged levels of the HP1-binding H3K9me3 mark. Biochemical analysis has further shown that H3S10ph can occur on the same histone tail as H3K9me3. Binding of HP1 to this dually H3K9me3S10ph modified H3 tail in vitro is highly reduced, and impairment of mitotic H3S10 Aurora B kinase activity retains HP1 on mitotic chromatin (Fischle et al. 2005; Hirota et al. 2005). Genetic studies on the cell cycle-dependent establishment of heterochromatin in *S. pombe* further sustain such a "methyl/phos switch" scenario. Later, it could be shown that similar mechanisms result in delocalization of a particular HP1 isoform away from heterochromatin in terminally differentiated plasma cells (Sabbattini et al. 2007).

Cross-talk between H3K9me and DNA methylation

DNA methylation, histone modification, chromatin structure, and gene silencing seem to be tightly interconnected. The first evidence for a link between H3K9me and DNA methylation came from genetic studies in *Neurospora*, where it was shown that mutations in DIM-5 abolish meC and relieve DNA methylation-mediated gene silencing (Tamaru and Selker 2001). A seemingly linear pathway could be worked out that involves recognition of the H3K9me3 mark laid down by DIM-5 by the adaptor HP1, which in turn recruits DIM-2 for cytosine methylation (Fig. 2A; Honda and Selker 2008). The pathway is a prime example for cross-talk by enzyme recruitment (see Fig. 1B). In plants, mutation of the *Arabidopsis* DNMT, MET1 causes severe changes in H3K9me2 and it was postulated, therefore, that CpG methylation is able to direct H3K9me2. Moreover, it could be demonstrated that H3K9me2, mediated by SUV4, directs non-CG methylation (Jackson et al. 2002). While HP1 is indispensable for DNA methylation in

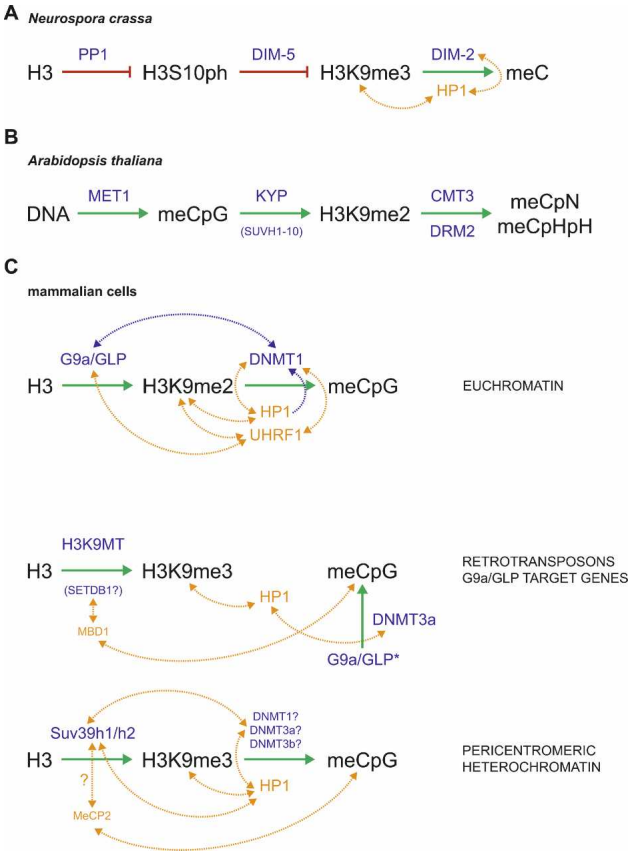

Figure 2. Cross-talk of H3K9me, H3S10ph, and DNA methylation in different organisms. (*A*) The new work by Adhvaryu and Selker (2008) places H310ph upstream of H3K9me and DNA methylation in *Neurospora*. (*B*) A two-step linear pathway of symmetric and nonsymmetric DNA methylation cross-talk with H3K9me2 is emerging in *Arabidopsis*. (*C*) In mammalian cells, distinct methylation stages of H3K9, different H3K9MTs, and various DNMTs seem to be involved in feedback signaling pathways between histones and DNA. The picture that is forming is one of a conversation full of subtle inflections, with multiple partners and mediators. Different pathways might be in place for different regions of the genome. (Blue) Chromatin-modifying enzymes; (orange) proteins binding chromatin marks; (green) positive cross-talk; (red) negative cross-talk; (blue arrows) stimulation of enzymatic activity; (orange arrows) binding/interaction; (G9a/GLP*) enzymatic inactive protein.

Neurospora, it is not required in *Arabidopsis* (Fuks 2005). In spite of many missing biochemical details, the available genetic data best support a "two-step" linear regulation of transcriptional silencing, in which CpG methylation directs H3K9 methylation and H3K9 methylation recruits non-CG methylation (Fig. 2B).

The picture emerging in mammalian cells is more complex. Evidence for cross-talk between DNA methylation and histone methylation in both directions has been found. On one side, treatment of certain cancer cell lines with the DNA demethylating drug 5-azaC results in reactivation of multiple silenced genes concomitant with a decrease in H3K9me (Wozniak et al. 2007). Similarly, DNMT1 and DNMT3b mutant mouse cells dis-

play altered H3K9 methylation patterns at heterochromatin and specific tumor suppressor loci (Fuks 2005). However, no clear reduction of global H3K9me was seen in DNMT1, DNMT3a, DNMT3b triple mouse knockout (KO) cells (Tsumura et al. 2006). On the other site, Suv39-deficient mouse embryonic fibroblast (MEF) cells show loss of H3K9me3, mislocalized DNMT3b, and absence of DNA methylation at pericentromeric heterochromatin, while H3K9me and DNA methylation persists in other regions; for example, at retrotransposons (Lehnertz et al. 2003). These regions are, however, affected in G9a-null mouse ES cells that show reduced CpG methylation in several euchromatic regions (Ikegami et al. 2007).

Over the last several years, biochemical insights into the interplay between DNA methylation and H3K9me in mammalian cells have been gained. The emerging picture does not resemble a unidirectional signaling pathway. It suggests, rather, complex cross-talk between mutually influencing chromatin marks and chromatin factors with many of the principles outlined in Figure 1 exemplified. An apparently reinforced system is emerging in euchromatic regions where DNMT1 acts together with G9a/GLP and HP1 to establish stable H3K9me and DNA methylation patterns (Fig. 2C). It could be shown that G9a/GLP and DNMT1 stimulate each other's activities, while G9a/GLP and HP1 enhance DNMT1 chromatin binding (Esteve et al. 2006; Smallwood et al. 2007). Further, HP1 also stimulates DNMT1 activity. Another player in this system seems to be UHRF1, which interacts with both DNMT1 and G9a/GLP (Sharif et al. 2007). Absence of UHRF1 causes DNMT1 mislocalization and global loss of DNA methylation (Bostick et al. 2007; Sharif et al. 2007). Since UHRF1 interacts with hemimethylated DNA, but also H3K9me3, it might be a crucial factor in the faithful transmission of both chromatin marks from one cell generation to the next.

A related, yet different system appears to be in play at retrotransposons. Recent work has indicated that the enzymatic activity of G9a/GLP is dispensable for transcriptional silencing of potentially active endogenous retroviruses, non-LTR retrotransposons, and euchromatic G9a/GLP target genes. H3K9me3, HP1 recruitment, and transcriptional silencing in this case seem to depend on another H3K9KMT, possibly SETDB1. Nevertheless, absence of G9a/GLP causes loss of DNMT3a and DNA methylation at target regions, which are both restored by catalytically dead G9a/GLP (Dong et al. 2008). The fact that 5-azaC relieves repression of G9a/GLP targets only in the absence of G9a/GLP enzymatic activity further supports mechanistic uncoupling of DNA methylation and H3K9me3 for gene silencing (Tachibana et al. 2008). Interestingly, SETDB1 can associate with the methyl-DNA-binding protein MBD1, and it was suggested that continued H3K9me at MBD1 target sequences and stable silencing depend on the periodic recruitment of this H3K9MT during replication (Sarraf and Stancheva 2004).

At pericentromeric heterochromatin recruitment of DNMTs to sites bearing the H3K9 methylation mark, might also be mediated by HP1, which seems capable of binding all mammalian DNMTs (Lehnertz et al. 2003; Fuks 2005). However, the cross-talking H3K9MT, in that case, appears to be Suv39, which was found to also interact with DNMT1 and 3a (Fuks 2005). While different reinforcing and potentially redundant mechanisms might be in place for cross-talk between DNA methylation and H3K9me for stable silencing of distinct regions of mammalian genomes, it has to be pointed out that so far no clear distinction between the establishment and the maintenance of DNA methylation and/or H3K9me chromatin marks have been made. Also, the different cross-talk mechanisms depicted in Figure 2C have not been tested in uniform experimental systems. It is therefore likely that these will be further refined and expanded in the near future.

Another layer of cross-talk—H3S10ph, H3K9me3, and DNA methylation

Already additional complexity in the cross-talk of H3K9me3, and DNA methylation is introduced by the work of Adhvaryu and Selker (2008). The investigators show that loss-of-function mutations in the phosphatase PP1 in *Neurospora* abolish DNA methylation at several—albeit not all investigated—regions of the genome. Since global levels of H3S10ph are elevated in the PP1 mutant fungi and as H3K9me3 is absent at the regions showing loss of DNA methylation, H3S10ph is obviously upstream of H3K9me3 and DNA methylation in this system (Fig. 2A). This interpretation is further supported by H3S10 mutation to nonphosphorylatable residues of the only H3 gene in *Neurospora* that causes similar distortion of H3K9me and DNA methylation. While the mechanisms of cross-talk between PP1 and H3S10ph on the one hand and H3K9me3 and meC on the other await further investigation, mutation of a DIM-5 residue that interacts with H3S10, interestingly, abolishes enzymatic activity in vitro. Also, H3 peptide methylation by DIM-5 is strongly reduced when Ser 10 is changed into more bulky, negatively charged glutamate (Rathert et al. 2008). The negative effects of H3S10ph onto H3K9me might therefore be a direct result of the substrate recognition and turnover properties of DIM-5 (see Fig. 1).

While the new work uncovers an additional layer in cross-talk of chromatin marks for gene silencing, it also raises interesting new questions. Is PP1 specifically targeted to regions of the genome that are to become H3K9me and meC? If so, what are the targeting mechanisms for PP1? What is the nature of the kinases mediating H3S10ph? Does H3S10ph act globally and thereby protect most of the genome from H3K9me and DNA methylation? How does this system interface with mitotic H3S10ph and its impact onto HP1 localization? Does the system need to be re-established during every cell cycle? Lastly, are similar mechanisms in play in higher eukaryotes?

That cross-talk between H3S10ph, H3K9me, and DNA methylation could be more complex is illustrated by recent studies of Aurora B kinase targeting. In mammalian cells, DNA methylation was found necessary to promote

Aurora B-driven phosphorylation of H3S10 at pericentromeric heterochromatin in G2 nuclei (Monier et al. 2007). Interestingly, H3S10ph at the onset of M-phase seems largely restricted to H3 molecules carrying the H3K9me mark (Fischle et al. 2005). The DNA methylation-dependent targeting of H3S10ph therefore might expand the "methyl/phos" switch cross-talk that evicts the adaptor HP1 from sites of H3K9me during mitosis. Without doubt, many layers of the chromatin modification regulatory network are yet to be uncovered.

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References

Adhvaryu, K.K. and Selker, E.U. 2008. Protein phosphatase PP1 is required for normal DNA methylation in *Neurospora*. *Genes & Dev.* (this issue). doi: 10.1101/gad.1738008.

Bao, X., Deng, H., Johansen, J., Girton, J., and Johansen, K.M. 2007. Loss-of-function alleles of the JIL-1 histone H3S10 kinase enhance position-effect variegation at pericentric sites in *Drosophila* heterochromatin. *Genetics* **176:** 1355–1358.

Bostick, M., Kim, J.K., Esteve, P.O., Clark, A., Pradhan, S., and Jacobsen, S.E. 2007. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* **317:** 1760–1764.

Collins, R.E., Northrop, J.P., Horton, J.R., Lee, D.Y., Zhang, X., Stallcup, M.R., and Cheng, X. 2008. The ankyrin repeats of G9a and GLP histone methyltransferases are mono- and dimethyllysine binding modules. *Nat. Struct. Mol. Biol.* **15:** 245–250.

Deng, H., Bao, X., Cai, W., Blacketer, M.J., Belmont, A.S., Girton, J., Johansen, J., and Johansen, K.M. 2008. Ectopic histone H3S10 phosphorylation causes chromatin structure remodeling in *Drosophila*. *Development* **135:** 699–705.

Dong, K.B., Maksakova, I.A., Mohn, F., Leung, D., Appanah, R., Lee, S., Yang, H.W., Lam, L.L., Mager, D.L., Schubeler, D., et al. 2008. DNA methylation in ES cells requires the lysine methyltransferase G9a but not its catalytic activity. *EMBO J.* **27:** 2691–2701.

Ebert, A., Schotta, G., Lein, S., Kubicek, S., Krauss, V., Jenuwein, T., and Reuter, G. 2004. Su(var) genes regulate the balance between euchromatin and heterochromatin in *Drosophila*. *Genes & Dev.* **18:** 2973–2983.

Esteve, P.O., Chin, H.G., Smallwood, A., Feehery, G.R., Gangisetty, O., Karpf, A.R., Carey, M.F., and Pradhan, S. 2006. Direct interaction between DNMT1 and G9a coordinates DNA and histone methylation during replication. *Genes & Dev.* **20:** 3089–3103.

Fischle, W., Wang, Y., and Allis, C.D. 2003. Histone and chromatin cross-talk. *Curr. Opin. Cell Biol.* **15:** 172–183.

Fischle, W., Tseng, B.S., Dormann, H.L., Ueberheide, B.M., Garcia, B.A., Shabanowitz, J., Hunt, D.F., Funabiki, H., and Allis, C.D. 2005. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. *Nature* **438:** 1116–1122.

Fuks, F. 2005. DNA methylation and histone modifications: Teaming up to silence genes. *Curr. Opin. Genet. Dev.* **15:** 490–495.

Grewal, S.I. and Elgin, S.C. 2007. Transcription and RNA interference in the formation of heterochromatin. *Nature* **447:** 399–406.

Hirota, T., Lipp, J.J., Toh, B.H., and Peters, J.M. 2005. Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin. *Nature* **438:** 1176–1180.

Honda, S. and Selker, E.U. 2008. Direct interaction between DNA methyltransferase DIM-2 and HP1 is required for DNA methylation in *Neurospora crassa*. *Mol. Cell. Biol.* **28:** 6044–6055.

Houben, A., Demidov, D., Caperta, A.D., Karimi, R., Agueci, F., and Vlasenko, L. 2007. Phosphorylation of histone H3 in plants—A dynamic affair. *Biochim. Biophys. Acta* **1769:** 308–315.

Ikegami, K., Iwatani, M., Suzuki, M., Tachibana, M., Shinkai, Y., Tanaka, S., Greally, J.M., Yagi, S., Hattori, N., and Shiota, K. 2007. Genome-wide and locus-specific DNA hypomethylation in G9a deficient mouse embryonic stem cells. *Genes Cells* **12:** 1–11.

Ivaldi, M.S., Karam, C.S., and Corces, V.G. 2007. Phosphorylation of histone H3 at Ser10 facilitates RNA polymerase II release from promoter-proximal pausing in *Drosophila*. *Genes & Dev.* **21:** 2818–2831.

Jackson, J.P., Lindroth, A.M., Cao, X., and Jacobsen, S.E. 2002. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* **416:** 556–560.

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.

Macdonald, N., Welburn, J.P., Noble, M.E., Nguyen, A., Yaffe, M.B., Clynes, D., Moggs, J.G., Orphanides, G., Thomson, S., Edmunds, J.W., et al. 2005. Molecular basis for the recognition of phosphorylated and phosphoacetylated histone h3 by 14–3–3. *Mol. Cell* **20:** 199–211.

McGinty, R.K., Kim, J., Chatterjee, C., Roeder, R.G., and Muir, T.W. 2008. Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature* **453:** 812–816.

Monier, K., Mouradian, S., and Sullivan, K.F. 2007. DNA methylation promotes Aurora-B-driven phosphorylation of histone H3 in chromosomal subdomains. *J. Cell Sci.* **120:** 101–114.

Nakatani, Y., Ray-Gallet, D., Quivy, J.P., Tagami, H., and Almouzni, G. 2004. Two distinct nucleosome assembly pathways: Dependent or independent of DNA synthesis promoted by histone H3.1 and H3.3 complexes. *Cold Spring Harb. Symp. Quant. Biol.* **69:** 273–280.

Papait, R., Pistore, C., Grazini, U., Babbio, F., Cogliati, S., Pecoraro, D., Brino, L., Morand, A.L., Dechampesme, A.M., Spada, F., et al. 2008. The PHD domain of Np95 (mUHRF1) is involved in large-scale reorganization of pericentromeric heterochromatin. *Mol. Biol. Cell* **19:** 3554–3563.

Rathert, P., Zhang, X., Freund, C., Cheng, X., and Jeltsch, A. 2008. Analysis of the substrate specificity of the Dim-5 histone lysine methyltransferase using peptide arrays. *Chem. Biol.* **15:** 5–11.

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.

Sabbattini, P., Canzonetta, C., Sjoberg, M., Nikic, S., Georgiou, A., Kemball-Cook, G., Auner, H.W., and Dillon, N. 2007. A

novel role for the Aurora B kinase in epigenetic marking of silent chromatin in differentiated postmitotic cells. *EMBO J.* **26:** 4657–4669.

Sarraf, S.A. and Stancheva, I. 2004. Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatin assembly. *Mol. Cell* **15:** 595–605.

Schulze, S.R. and Wallrath, L.L. 2007. Gene regulation by chromatin structure: Paradigms established in *Drosophila melanogaster*. *Annu. Rev. Entomol.* **52:** 171–192.

Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A., Shinga, J., Mizutani-Koseki, Y., Toyoda, T., Okamura, K., et al. 2007. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* **450:** 908–912.

Shogren-Knaak, M.A., Fry, C.J., and Peterson, C.L. 2003. A native peptide ligation strategy for deciphering nucleosomal histone modifications. *J. Biol. Chem.* **278:** 15744–15748.

Smallwood, A., Esteve, P.O., Pradhan, S., and Carey, M. 2007. Functional cooperation between HP1 and DNMT1 mediates gene silencing. *Genes & Dev.* **21:** 1169–1178.

Tachibana, M., Matsumura, Y., Fukuda, M., Kimura, H., and Shinkai, Y. 2008. G9a/GLP complexes independently mediate H3K9 and DNA methylation to silence transcription. *EMBO J.* **27:** 2681–2690.

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

Taverna, S.D., Li, H., Ruthenburg, A.J., Allis, C.D., and Patel, D.J. 2007. How chromatin-binding modules interpret histone modifications: Lessons from professional pocket pickers. *Nat. Struct. Mol. Biol.* **14:** 1025–1040.

Tsumura, A., Hayakawa, T., Kumaki, Y., Takebayashi, S., Sakaue, M., Matsuoka, C., Shimotohno, K., Ishikawa, F., Li, E., Ueda, H.R., et al. 2006. Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. *Genes Cells* **11:** 805–814.

Vaillant, I. and Paszkowski, J. 2007. Role of histone and DNA methylation in gene regulation. *Curr. Opin. Plant Biol.* **10:** 528–533.

Vakoc, C.R., Mandat, S.A., Olenchock, B.A., and Blobel, G.A. 2005. Histone H3 lysine 9 methylation and HP1γ are associated with transcription elongation through mammalian chromatin. *Mol. Cell* **19:** 381–391.

Volkel, P. and Angrand, P.O. 2007. The control of histone lysine methylation in epigenetic regulation. *Biochimie* **89:** 1–20.

Winter, S., Simboeck, E., Fischle, W., Zupkovitz, G., Dohnal, I., Mechtler, K., Ammerer, G., and Seiser, C. 2008. 14–3–3 proteins recognize a histone code at histone H3 and are required for transcriptional activation. *EMBO J.* **27:** 88–99.

Woo, H.R. and Richards, E.J. 2008. Signaling silence—Breaking ground and spreading out. *Genes & Dev.* **22:** 1719–1723.

Wozniak, R.J., Klimecki, W.T., Lau, S.S., Feinstein, Y., and Futscher, B.W. 2007. 5-Aza-2′-deoxycytidine-mediated reductions in G9A histone methyltransferase and histone H3 K9 di-methylation levels are linked to tumor suppressor gene reactivation. *Oncogene* **26:** 77–90.

Zhang, W., Deng, H., Bao, X., Lerach, S., Girton, J., Johansen, J., and Johansen, K.M. 2006. The JIL-1 histone H3S10 kinase regulates dimethyl H3K9 modifications and heterochromatic spreading in *Drosophila*. *Development* **133:** 229–235.