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SET for life: biochemical activities and biological functions of SET domain-containing proteins

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Abstract

SET domain-containing proteins belong to a group of enzymes named after a common domain that utilizes the cofactor S-adenosyl-L-methionine (SAM) to achieve methylation of its substrates. Many SET domain-containing proteins have been shown to display catalytic activity towards particular lysine residues on histones, but emerging evidence also indicates that various non-histone proteins are specifically targeted by this clade of enzymes. Here, we summarize the most recent findings on the biological functions of the major families of SET domain-containing proteins catalyzing the methylation of histones 3 on lysines 4, 9, 27, and 36 (H3K4, H3K9, H3K27, and H3K36) and histone 4 on lysine 20 (H4K20) as well as candidates that have been reported to regulate non-histone substrates.

Keywords

SET domain-containing proteins; histone lysine methylation; non-histone substrates

SET domain-containing proteins

SET domain-containing proteins exist in all eukaryotes studied to date. This protein family is characterized by an approximately 130 amino-acid-long domain called the SET domain, which was named after the *Drosophila* proteins **S**uppressor of variegation 3-9 (Su(var)3-9), **E**nhancer of zeste (E(z)), and **T**rithorax (Trx). The SET domain possesses catalytic activity towards the ε-amino group of lysine residues. Depending on the context and their biochemical properties, SET domain-containing proteins are able to mono-, di-, or trimethylate their lysine substrates by utilizing the cofactor S-adenosyl-L-methionine (SAM). In vivo, lysine methylation is often dynamically regulated by the opposing actions of lysine methyltransferases and lysine demethylases. Initially reported to catalyze the methylation of histones, it has now become increasingly clear that SET domain-containing proteins also target many non-histone substrates, some of which constitute regulators of signaling pathways, transcription factors, and tumor suppressors (Tables 1-3, Boxes 1-2). The grouping of SET domain-containing proteins based on sequence similarity of their SET domains often closely reflects an already reported specificity for certain substrates (Figure 1, showing human SET domain-containing proteins). Here, we provide an overview on the biological functions of the major groups of SET domain-containing histone lysine

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methyltransferases (KMTs) based on their substrate specificity towards histones. Apart from affecting chromatin states either by directly methylating histones (Figures 1-2) and thus altering the chromatin environment to enhance or suppress the binding of co-factors, KMTs have also been reported to target non-histone proteins (Table 1, Box 1). Importantly, many other SET domain-containing proteins are only known to methylate non-histone substrates and do not appear to target histones directly (Tables 2-3, Box 2). Furthermore, the SET domain does not generally exist as an independent entity, as in many proteins it co-occurs with multiple other protein domains (Figure 1). Some SET domain-containing proteins are found in complexes or interact with proteins that regulate their target specificity and catalysis (Figure 3).

Histone H3K4 methyltransferases

Substrate specificity

H3K4 KMTs are conserved in plants, yeast, *Drosophila*, and mammals. Yeast Set1 is the sole enzyme that catalyzes the mono-, di-, and trimethylation of H3K4 [1, 2]. In *Drosophila*, yeast Set1 is represented by three homologous proteins: Set1, Trithorax (Trx), and Trithorax-related (Trr). Mammals contain six yeast Set1-related proteins with SET1A and SET1B (related to *Drosophila* Set1); MLL1 and MLL2 (related to *Drosophila* Trx); and MLL3 and MLL4 (related to *Drosophila* Trr) [1] (Figure 1). *Drosophila* Set1 constitutes a major di- and trimethyltransferase and this function is redundantly shared by mammalian SET1A/B [3-6]. Although the loss of Trx/MLL1/MLL2 does not result in bulk changes of any H3K4 methyl mark in most contexts, Mll2 is globally required for H3K4 trimethylation in oocytes [3, 7, 8]. Trr (and based on homology we predict mammalian MLL3/4 in a redundant fashion) is a major H3K4 monomethyltransferase [9]. Besides Trr, which is only homologous to the C-terminal portion of mammalian MLL3/4, *Drosophila* also contains another protein, LPT, with homology to the N-terminus of MLL3/4, and like Trr, LPT is also required for bulk H3K4 monomethylation [3, 9, 10]. Thus in *Drosophila*, two proteins, Trr (bearing the catalytic SET domain) and LPT, constitute the functional homolog of mammalian MLL3/4. All three *Drosophila* H3K4 KMTs are essential for viability [5, 11, 12], and *Mll1* and *Mll2* knockout mice show embryonic lethality confirming the importance of these genes in development [13, 14]. However, selective deletion of the *Mll1* SET domain yields viable offspring albeit with various defects [15]. This suggests that the function of MLL1 and possibly most SET domain-containing proteins cannot simply be reduced to their methyltransferase activity as many of these enzymes contain important domains that might function independently of the SET domain.

With the yeast Set1 target Dam1, only one non-histone substrate among the H3K4 KMTs has been reported to date (Table1, Box 1). Additionally, SETD7 (SET7/SET9) has been demonstrated to be an H3K4 monomethyltransferase. However, SETD7 only displays limited activity on nucleosomal substrates, making it an unlikely candidate for H3K4 methylation in vivo. In fact, the identification of many non-histone substrates of SETD7 implies that they represent the functionally relevant SETD7 targets in vivo (Table 2, Box 2) [16]. Although *Drosophila* Ash1/mammalian ASH1L and MLL5 have been described as H3K4 KMTs [17-19], these initial reports could not be confirmed by others [20-23]. Instead, Ash1/ASH1L constitute an H3K36 dimethyltransferase; and UpSET, the *Drosophila* homolog of MLL5, restricts chromatin accessibility by binding to a Rpd3/Sin3-containing histone deacetylase complex [22]. Finally, SMYD3 can methylate H3K4, H4K5, and H4K20, but also targets non-histone substrates such as FLT1 (Table 3) [24-27]. Considering that SMYD3 does not display distinct substrate specificity on histones and also targets non-histone substrates, it is unlikely that it represents an H3K4 KMT in vivo.

Protein complexes (Figure 3)

In order to exert its catalytic activity as an H3K4 KMT, yeast Set1 requires the presence of additional subunits, which together with the catalytic SET domain-containing enzyme form a complex named COMPASS (Complex of Proteins Associated with Set1). In yeast, these subunits are Cps60, Cps50, Cps40, Cps35, Cps30, Cps25, and Cps15 [1]. The *Drosophila* and mammalian H3K4 KMTs exist in COMPASS-like complexes and all share the core subunits Ash2 in *Drosophila*/ASH2L in mammals (related to Cps60); RBBP5 (related to Cps50); Wds in *Drosophila*/WDR5 in mammals (related to Cps30); Dpy-30L1 in *Drosophila*/DPY30 in mammals (related to Cps25); and Hcf in *Drosophila*/HCFC1 in mammals (no yeast homolog). Besides these common subunits, each of the three "subbranches" contains complex-specific subunits. The *Drosophila* Set1 and the mammalian SET1A/B complexes share Cxxc1/CXXC1 (related to Cps40); Wdr82/WDR82 (related to Cps35); the Trx and MLL1/2 complexes Mnn1 in *Drosophila*/MEN1 in mammals; and the Trr and MLL3/4 complexes Ncoa6/NCOA6, Pa1/PA1, Ptip/PTIP, and Utx/UTX [1, 3].

Biochemical reconstitution followed by three dimensional cryo-electron microscopy of the yeast SET domain along with the core components Cps60, Cps50, Cps30, and Cps25 revealed that the yeast COMPASS "core" exists in a Y-shaped configuration with Cps50/Cps30 forming the top two adjacent lobes of the "Y" and Cps60/Cps25 constituting the stem. The localization of the SET domain at the juncture of Cps50/Cps30 and Cps60/Cps25 results in a channel that can only be accessed by a flexible peptide suggesting that COMPASS can only add methyl groups to amino acid residues that are located very close to the start or end of a protein [28]. Similarly, WDR5, RBBP5, and ASH2L pose fundamental components for the mammalian COMPASS-like complexes [28, 29]. WDR5 is essential for the assembly of all mammalian COMPASS-like complexes through its interaction with the WDR5 interaction (Win) motif located just N-terminally of the SET domain; and basic and acidic patches in Cps50/RBBP5 are required for its interaction with Set1/SET1A/SET1B [30-33]. Additionally, interaction between WDR5 and RBBP5 is crucial for complex assembly and activity [34, 35]. Both ASH2L and RBBP5 together with the catalytic SET domain are able to directly contact the substrate SAM to form a joint catalytic center [36]. The DNA-binding properties of the ASH2L N-terminus are necessary for optimal H3K4 KMT activity and transcriptional output of target genes [37, 38].

Transcription and recruitment of H3K4 KMTs

Set1/SET1A/SET1B are mainly involved in regulating H3K4 tri- and dimethylation on promoters and bodies of actively transcribed genes [1, 6]. An E2/E3 ubiquitin ligase module consisting of RAD6/BRE1 directly mediates H2B monoubiquitination and is required for proper implementation of H3K4 di- and trimethylation by Set1/SET1A/SET1B [1]. The Cps35/WDR82 subunit of COMPASS interacts with chromatin and COMPASS in an H2B monoubiquitination dependent manner and is required for H3K4 di- and trimethylation similar to both RAD6 and BRE1 [1, 39-42]. In yeast, asymmetric H3 arginine 2 (H3R2) dimethylation prevents the recruitment of Cps40, and thus, globally affects H3K4 trimethylation via Set1 [43]. Similar results were observed in mammals but have been attributed to MLL1 recruitment on *Homeobox* (*HOX)* genes [44, 45]. Therefore, it is possible that asymmetric H3R2 dimethylation might interfere with the recruitment of various COMPASS-like complexes in mammals. In mammals, the Set1a/b complex-specific subunit Cxxc1 (Cps40 in yeast) is also recruited to unmethylated CpG islands to implement H3K4 trimethylation; and artificial CpG sequences in the absence of promoters are able to recruit Cxxc1 and induce H3K4 trimethylation [46]. However, depending on the context, Cxxc1 is not only required to implement H3K4 trimethylation on promoter-proximal sequences, but also prevents introduction of H3K4 trimethylation on promoter-distal elements [47]. How this is achieved is currently unknown.

Drosophila studies have described Trx as a positive regulator of *Hox* genes and important developmental genes. This was also subsequently confirmed in mammals [1]. Indeed, while Mll1 is only required for the H3K4 trimethylation of a subset of *Hox* genes, *Men1* knockout results in a dramatic loss of H3K4 trimethylation and gene expression on all *Hox* gene clusters in mouse embryonic fibroblasts providing evidence that Mll1/2 function redundantly on *Hox* genes and specifically target this gene class [8]. In yeast, the RNA Polymerase II (Pol II) interacting Paf1 complex has a dual function by controlling H2B monoubiquitination through Rad6/Bre1 and by providing a landing pad for Pol II to promote Set1-dependent H3K4 di- and trimethylation. This role has been confirmed for MLL1 on *HOX* genes in mammals as well [1, 48-51]. Long noncoding (nc) RNAs could also play a potential role in targeting the Trx/MLL1/MLL2 COMPASS-like complexes. For example, the ncRNA *HOTTIP* is transcribed from the 5′ tip of the human *HOXA* locus and binds WDR5 to recruit MLL1 across the *HOXA* cluster and the ncRNA *Mistral* activates transcription of *Hoxa6* and *Hoxa7* by recruiting Mll1 in mouse ES cells [52, 53].

Trr/MLL3/MLL4 interact with hormone receptors and are recruited to the promoters of target genes where they induce H3K4 trimethylation and transcription in *Drosophila* and mammals [1, 54-57]. The process of nuclear receptor signaling via Trr in *Drosophila* is highly regulated. In the absence of hormone (ecdysone), the ecdysone receptor resides in the cytoplasm, but upon binding to ecdysone, forms a heterodimer with the hormone receptor Ultraspiracle. The heterodimeric complex then translocates together with Trr into the nucleus to activate ecdysone-inducible genes [58]. Mammalian MLL3/4 are also involved in the P53-mediated DNA damage response. By binding to P53 the MLL3/4 complex-specific subunit NCOA6 recruits MLL3/4 COMPASS-like complexes to the promoters of the P53 target genes to catalyze H3K4 trimethylation and induce transcription [59].

Functions of MLL1 during the cell cycle

The protein levels of MLL1 are tightly controlled during the cell cycle and peak at the G1/S and G2/M transition. SCF and APC E3 ubiquitin ligase complexes ensure that MLL1 is degraded by the proteasome during the S and M phase, respectively [60]. Apart from controlling cell cycle regulators in G1, MLL1 also plays roles in origin of replication firing during S phase. Upon DNA damage, the checkpoint kinase ATR phosphorylates MLL1 on serine 516 preventing its degradation by SCF. MLL1 accumulation on chromatin prevents loading of the essential pre-replication complex component CDC45 which results in replication blockage [61]. In contrast to most transcription factors, MLL1 remains associated with mitotic chromosomes during M phase. Genes that are exclusively bound by MLL1 in mitosis are particularly highly expressed during interphase. Thus, MLL1 is thought to provide a “priming” platform for highly expressed S phase genes [60, 62].

Trr/MLL3/MLL4 and enhancer-associated H3K4 monomethylation

H3K4 monomethylation is a mark that is broadly distributed across the genome of *Drosophila* and mammals and enriched on the bodies of actively transcribed genes and cis-regulatory elements called enhancers [63, 64]. Although *Drosophila* Trr and its mammalian homologs MLL3/4 have been described as promoter-proximal H3K4 trimethyltransferases in hormone receptor-mediated transcription, studies based on a global H3K4 monomethylation reduction in *Utx* and *trr* mutant tissue show that in fact Trr constitutes a major H3K4 monomethyltransferase. Trr localizes to promoters but is also enriched on promoter-distal elements together with the Trr complex-specific subunits LPT and Utx, the histone acetyltransferase CBP, and broad domains of H3K4 monomethylation. Trr is able to convey enhancer-mediated activation of the *cut* locus in vivo and knockdown of Trr results in reduced H3K4 monomethylation, H3K27 acetylation, and a concomitant increase of H3K27 trimethylation on enhancers. This suggests that Trr/MLL3/MLL4 COMPASS-like

complexes also function in enhancer-mediated processes by providing a hub for H3K27 demethylation of inactive/"poised" enhancers via Utx/UTX, followed by H3K4 monomethylation by Trr/MLL3/MLL4, and subsequent acetylation by CBP/p300 to create an activated enhancer state [9, 65].

Histone H3K9 methyltransferases

Substrate specificity

SUV39H1 was the first SET domain-containing protein reported to contain methyltransferase specificity in general and towards H3K9 in particular [66, 67]. Subsequently, the list of H3K9 KMTs has been expanded considerably, and in mammals, now includes SUV39H1, SUV39H2, G9A, GLP, SETDB1, SETDB2, PRDM3, and PRDM16 [66-68] (Figures 1-2). PRDM3 and PRDM16 are members of the PRDM family and contain an N-terminal PR domain, which is closely related to the SET domain [68, 69]. With a panoply of possible candidates in this group, it has been difficult to clearly separate state-specific functions in H3K9 methylation for any particular enzyme. It appears that the mono-, di-, and trimethylated states of H3K9 are in many cases either regulated redundantly or context-specifically based on the catalytic function, recruitment mechanisms to chromatin, and potentially varying expression patterns of the respective enzymes. SUV39H1/2 mediate the bulk of H3K9 trimethylation [70]. They preferentially localize to pericentric heterochromatin and other regions that contain repetitive DNA elements such as telomeres. On telomeres SUV39H1/2 also mediate dimethylation of H3K9 [67, 71]. In contrast, G9A and GLP are euchromatic H3K9 methyltransferases and act in a heterodimeric complex together with WIZ, a multi-zinc finger protein, to globally achieve mono-and dimethylation of H3K9 [66, 70, 72-74]. SETDB1 has also been ascribed bulk di- and trimethyltransferase activity and directs repression of euchromatic genes with the chromatin-associated factor ATF7IP (MCAF, AM), aiding in the conversion from the H3K9 dimethylated state to the trimethylated state [75]; whereas SETDB2 affects H3K9 trimethylation at pericentric heterochromatin [76]. How SUV39H1, SUV39H2, G9A, GLP, and SETDB1 can display overlapping state-specific, bulk H3K9 methylation changes is currently not clear. One explanation might be the co-occurrence of SUV39H1, G9A, GLP, and SETDB1 in a mega-complex [77]. Therefore, removing the function of one H3K9 KMT might also result in the loss of activity of the other candidates on common chromatin targets. In contrast, Prdm3 and Prdm16 function redundantly as cytoplasmic H3K9 monomethyltransferases [78]. Other histone substrates for H3K9 KMTs such as linker histone H1 mono- and dimethylation on lysine 26 and H3K27 and H3K56 methylation by G9A and GLP have been described [79-82] and several non-histone targets for H3K9 KMTs have been reported (Box 1).

Drosophila orthologs exist for all of the mammalian H3K9 KMTs including Su(var)3-9 for SUV39H1/2 [83], G9a for G9A/GLP [84], Eggless for SETDB1/2 [85, 86], and Hamlet for PRDM3/16. H3K9 KMT activities have been reported for *Drosophila* Su(var)3-9, G9a, and Eggless, but not for Hamlet. Generally, the *Drosophila* studies confirm the mammalian findings with occasional discrepancies as to the state-specific effects of the respective enzymes on H3K9 methylation [83, 85, 87, 88]. In *Drosophila*, Eggless and Hamlet are the only H3K9 KMT members that are essential for viability [85, 89], which is in contrast to the mammalian system where Suv39h1/2, G9a, Glp, Setdb1, Prdm3, and Prdm16 are all required for proper embryonic development. However, some *Suv39h1/2* double-knockout mice survive into adulthood [72, 73, 90-93].

Protein complexes (Figure 3)

In contrast to H3K4 and H3K27 KMTs which form stereotypic stable complexes, the H3K9 KMTs appear to form more transient interactions or have the ability to change based on the context and local environment and requirements. For example, SETDB1 is recruited to euchromatic regions by autosumoylated TRIM28 (KAP1) to silence transcription in an HP1α-dependent mechanism [94-96]. SETDB1 has been described to form an S phase-specific protein complex with the methyl-CpG-binding protein MBD1 and the chromatin assembly factors CHAF1A (CAF1P150), CHAF1B (CAF1P60), and RBBP4 to achieve H3K9 methylation during replication [97, 98]. Cell-type specific recruitment of this TRIM28/CHAF1A/CHAF1B/RBBP4-containing SETDB1 complex is mediated by zinc finger transcription factors such as ZNF274 [99]. MBD1 also forms a SETDB1-containing complex with ATF7IP1 and ATF7IP2 to induce heterochromatin formation via HP1α, -β, and -γ [100]. More recently, a subset of SUV39H1, SETDB1, G9A, and GLP has been shown to exist in a multi-meric mega-complex that targets satellite repeats and regulates G9A target genes [77].

Repressive domains of H3K9 tri- and dimethylation

Heterochromatic regions such as pericentric chromatin and other regions contain repetitive DNA elements that are characterized by strong enrichment of H3K9 trimethylation, which is important in maintaining genome stability. SUV39H1/2-directed pericentric chromatin formation affects chromosomal stability and is involved in regulation of telomere length [71, 90]. The H3K9 di-and trimethyl binder HP1α interacts with SUV39H1 and has been implicated in a self-enforcing heterochromatin-spreading mechanism in which an initial H3K9 methylation event by SUV39H1 is enforced by the recruitment of HP1α and the subsequent spreading of the H3K9 trimethyl mark through continued SUV39H1 activity [66]. Likewise, loss of the *Drosophila* SUV39H1/2 ortholog Su(var)3-9 results in spontaneous heterochromatic DNA damage which is accompanied by translocation defects [101]. Interestingly, H3K9 monomethylation by PRDM3/16 may be a prerequisite for further H3K9 trimethylation by SUV39H1/2 as co-depletion of the murine H3K9 monomethyltransferases Prdm3/16 results in heterochromatic defects that resemble those of Suv39h1/2 loss including a bulk reduction in H3K9 trimethylation and derepressed satellite transcription [78]. However, because PRDM3 interacts with SUV39H1, it is possible that the role of PRDM3 and/or PRDM16 in H3K9 trimethylation and heterochromatic silencing might be mediated by this physical interaction and not their H3K9 monomethylation activity per se [102-104]. A recent study in *C. elegans* supports the importance of H3K9 methylation in repeat-rich heterochromatin to maintain interaction with the nuclear lamina as depletion of MET-2, the *C. elegans* homolog of SETDB1 and SET-25 another H3K9 trimethyl-specific KMT, results in chromosome detachment from the nuclear periphery [105].

The mammalian genome also contains large megabase-sized domains that are enriched for H3K9 di- and trimethylation and nuclear lamins, but are distinct from the previously described constitutive heterochromatin. They emerge during differentiation, localize to the nuclear periphery, and are thought to prevent the transcription of gene blocks that need to be silenced [67, 106, 107]. G9a represses genes within these domains of facultative heterochromatin, but is not required for their localization to the nuclear periphery [66, 108]. HP1α interacts with the G9A and GLP complexes, which requires the automethylation of G9A on a histone-like sequence [109, 110]. Both the chromodomain of HP 1α and the ankyrin repeats in G9A/GLP can bind H3K9 dimethyl marks [111]. Analogous to the spreading mechanism of HP1α and SUV39H1 in constitutive heterochromatin, this allows spreading of H3K9 dimethylation over large domains [66].

Transcriptional repression (and activation)

Various proteins, including mainly transcription factors and corepressors, have been proposed to interact and/or recruit H3K9 KMTs, and thus, provide a possible rational for target specificity. Generally, most studies suggest a function in transcriptional repression for the respective H3K9 KMTs. Gene repression by H3K9 KMTs usually correlates with increased H3K9 methylation on promoters and in some cases has been demonstrated to depend directly on the catalytic activity of the enzyme. Transcriptional repression by H3K9 KMTs is required in many developmental and physiological contexts including neurogenesis and the hypoxia and stress response (Box 3). However, evidence also exists for a direct involvement of some H3K9 KMTs in transcriptional activation by either RNA Polymerase I or II [112-114].

Transcriptional repression of cell cycle components

BCL1 1B (CTIP2) affects cell cycle progression by recruiting SUV39H1 to the cell cycle inhibitor gene *P21*[115]. Similarly, G9A has been reported to be required for repression of *P21*, which is also a known P53 target [116]. Therefore, not surprisingly SUV39H1 and GLP have been shown to regulate the P53 pathway. The E3 ubiquitin ligase MDM2 interacts with SUV39H1 and GLP to drive formation of P53/SUV39H1 and P53/GLP “complexes” that are able to repress *P21* transcription during P53-mediated stress response [117]. Conversely, inhibition of P53 target genes via SUV39H1 and GLP can be relieved by binding of the P53 activator CDKN2A (ARF) to MDM2 [117]. Furthermore, GLP also methylates P53 directly on lysine 373 resulting in the inhibition of P53 activity (Table 1). This inhibition can be released by the P53 activator CDKN2A [117]. Evidence for silencing of another cell cycle inhibitor, P16, by SUV39H2 functionally connects the Polycomb machinery with H3K9 KMTs. Silencing of the *P16* gene is thought to be initiated by the Polycomb homolog CBX7. CBX7-mediated recruitment of SUV39H2 and the subsequent trimethylation of H3K9 then results in further compaction of the *P16* locus [118].

Histone H3K27 methyltransferases

Substrate specificity

Drosophila Enhancer of zeste (E(z)) is a member of the Polycomb group of proteins functioning in the negative regulation of genes that are crucial for the developmental patterning of organisms – the so called homeotic genes [119-121]. Later, it was shown that E(z) and its mammalian homologs, EZH1 and EZH2 (Figures 1-2), are the catalytically active components of a protein complex named Polycomb repressive complex 2 (PRC2), which is able to methylate H3K27 [119] (Figure 2). *E(z)* null mutant larvae in *Drosophila* lose all H3K27 mono-, di-, and trimethylation [122]. EZH2 is responsible for the bulk di- and trimethylation of H3K27 in mammals but has also been described to mono- and dimethylate linker histone H1 on lysine 26 [123] and to methylate non-histone targets (Table 1, Box 1). EZH1 is a weaker H3K27 di- and trimethyltransferase and is more highly expressed in nonproliferative cells [124]. Similar to *Drosophila* E(z), EZH1/2 function redundantly to implement all three H3K27 methylation states [125]. Both *Drosophila E(z)* and mouse *Ezh2* are essential genes (*Ezh1* knockout mice have not been reported to date) highlighting the importance of H3K27 KMTs in development [126, 127].

Protein complexes (Figure 3)

Like the COMPASS family of H3K4 KMTs E(z)/EZH1/EZH2 require the presence of other proteins to exert catalytic activity towards H3K27. The core subunits are comprised of E(z), Su(z), Esc/Escl, and Nurf55 in *Drosophila* and EZH1/2, SUZ12, EED, and RBBP4/7 in mammals [119]. EED exists in four isoforms of which the largest assembles in a particular

PRC2 complex that is able to mono- or dimethylate histone H1 on lysine 26 resulting in transcriptional repression [123]. EED in the core PRC2 complex appears to be required for H3K27 di- and trimethylation spreading as binding of the EED/Esc C-terminus to trimethylated H3K27 is required for the allosteric activation of PRC2 [128, 129]. In contrast, H3K4 trimethylation and H3K36 di- and trimethylation allosterically inhibit PRC2 via the SUZ12/Su(z)12 C-terminus [130, 131]. SUZ12 is also capable of "sensing" its chromatin environment and binds to an amino acid stretch (aa 31-42) on H3 of neighboring nucleosomes. Optimal H3K27 methyltransferase activity is only achieved if the neighboring nucleosome is close enough to be contacted by SUZ12. Local chromatin compaction preceding H3K27 methylation was illustrated for the gene Cyp26a1 [132]. The recent publication of the molecular architecture of human PRC2 supports these roles of EED/Esc and SUZ12/Su(z)12, and localizes the points of interaction of the PRC2 core subunits with each other in more detail [133].

Modulation of PRC2 activity and recruitment

Other accessory factors have been described that either modulate PRC2 activity and/or are required for the context-specific recruitment of PRC2 to chromatin [119]. These include Jing, Pcl, and Jarid2 in *Drosophila*[134-136] and AEBP2, PCL1-3 (PHF1, MTF2, PHF19), and JARID2 in mammals [137-141].

Beside the histone modifications mentioned above, H3K27 acetylation is implemented by CBP/p300, and by nature of modifying the same residue, is mutually exclusive with H3K27 trimethylation on PRC2 target genes [142-144]. H3 serine 28 phosphorylation (H3S28) constitutes another opposing histone mark to H3K27 trimethylation. MSK1 and MSK2 have been identified as the responsible kinases that phosphorylate H3S28 resulting in a transitional H3K27 trimethylation-S28 phosphorylation double modification, which subsequently is resolved to H3K27 acetylation-S28 phosphorylation. H3K27 trimethylation-S28 phosphorylation is thought to displace PRC2 to recruit transcriptional activators resulting in derepression of PRC2 target genes [145, 146].

In *Drosophila*, specific sequences called Polycomb response elements (PREs) are sufficient to recruit PRC2 via its interaction with PRE-binding transcription factors, supporting evidence for this mechanism in the mammalian system is rather sparse [147-150]. However, in mammals, PRC2 interacts with many ncRNAs, some of which directly recruit PRC2 in cis or trans to establish H3K27 trimethylation and transcriptional silencing [151, 152]. For example, the ncRNA *Xist* directly recruits PRC2 to the female X-chromosome during X-chromosome inactivation [153, 154], and the ncRNA *HOTAIR* which is expressed from a region of the human *HOXC* locus silences a portion of the *HOXD* cluster in trans via recruitment of PRC2 [155, 156]. Interestingly, the phosphorylation of EZH2 on threonine 350 (threonine 345 in mice) by the cell cycle-regulated Cyclin-dependent kinase 1 (CDK1) increases its binding to *HOTAIR* [157]. Another study reports that phosphorylation of human EZH2 on threonine 350 results in the increased recruitment of PRC2 and the hypersilencing of many PRC2 target loci [158]. However, matters are further complicated by the finding that phosphorylation of EZH2 on threonine 492 (threonine 487 in mice) appears to destabilize the PRC2 complex and that the phosphorylation of mouse Ezh2 on threonine 345 and 487 promotes Ezh2 ubiquitylation and subsequent degradation via the proteasome [159, 160]. In summary, this implies that phosphorylation of EZH2 affects recruitment of PRC2 either through ncRNAs or other factors to regulate PRC2 target gene expression in a cell cycle-dependent manner, but that at the same time, mechanisms exist that allow for the timely degradation of phosphorylated EZH2 as cells transition from one phase of the cell cycle to the next. Other kinases such as AKT and mitogen-activated protein kinase P38 have also been reported to phosphorylate EZH2 on serine 21 and threonine 372, respectively

[161, 162]. For example, phosphorylation of EZH2 on serine 21 by AKT negatively regulates EZH2 activity and p38 activation in satellite cells promotes the interaction of Ezh2 with the transcription factor Yy1 via phosphorylation on threonine 372 to repress *Pax7* transcription [161, 162].

miRNAs provide yet another mechanism by which EZH2 protein levels are controlled. They bind to the UTRs of the *EZH2* transcripts to limit the transcriptional output of EZH2. A negative feedback loop between PRC2 and miRNA loci ensures that this process remains tightly controlled [163-165]. As miRNAs are often expressed tissue specifically, they might provide one explanation of how EZH2 levels can be regulated in specific cell types.

Transcriptional regulation

H3K27 monomethylation tends to be associated with constitutive heterochromatin (along with H3K9 trimethylation) and inactive/poised enhancers, whereas H3K27 di- and trimethylation occur largely on facultative heterochromatin, but can also be found on intergenic and subtelomeric chromatin regions [119, 166]. H3K27 trimethylation is often enriched over large domains that can comprise up to 10 kb in *Drosophila* and 100 kb in mammals. Mammalian studies have shown that these domains increase in size upon differentiation resulting in even stronger transcriptional silencing [167]. However, trimethylated H3K27 also localizes more distinctly on transcriptionally inactive genes outside these domains [168]. This is in agreement with the finding that PRC2 has been reported to maintain gene repression of *HOX* genes and many other developmentally regulated genes [119]. Nonetheless, Pol II phosphorylated on serine 5 (a mark for transcription initiation) can be detected on a significant number of PRC2-targeted promoters. Additionally, a *Drosophila* study in *esc* mutant embryos reports augmented levels of Pol II on many PRC2 target genes suggesting a role for PRC2 in Pol II pausing [119, 169]. Indeed, some PRC2-repressed genes that are bivalently marked by H3K4 and H3K27 trimethylation in primary T and ES cells are characterized by Pol II recruitment and the transcription of 50-200 nucleotide long short RNAs. These short RNAs form stem-loop structures which interact with SUZ12 and are thought to result in gene repression in cis [170].

However, increasing evidence suggests that PRC2 can also function in active transcriptional processes. For example, H3K27 mono- and trimethylation can be enriched on actively transcribed genes as well [166, 171], and PRC2 (Suz12) is recruited to differentiation-activated genes in mouse ES cells [172]. Ezh1 overlaps with the H3K4 trimethyl mark on promoters of actively transcribed genes in mouse myoblasts [173]. Furthermore, Ezh1 depletion resulted in reduced Pol II occupancy and delayed transcriptional activation supporting a role for PRC2 in transcriptional elongation [173]. *Drosophila* studies support this idea as E(z) and Jarid2 loss have been described to result in repression of some PRC2-bound genes [134].

EZH2 can also activate genes independently of the PRC2 complex which potentially occurs under conditions where increased levels of EZH2 cannot be bound by other PRC2 core components such as in cancer cells. For example, EZH2 functions as a transactivator of the NF-κB pathway by interacting with RELA and RELB and has been shown to positively integrate the estrogen receptor α (ERα) and Wnt signaling pathways by binding to ERα and the Wnt pathway components TCF and β-Catenin. In both cases, this occurs independently of its methyltransferase activity [174, 175]. Furthermore, in a SET domain-dependent process EZH2 was found to be phosphorylated on lysine 21 by AKT leading to Androgen receptor (AR) binding and activation of AR target genes [176].

Histone H3K36 methyltransferases

Substrate specificity

In mammalian cells, H3K36 KMTs include SET2 (SETD2), NSD1, NSD2 (WHSC1, MMSET), NSD3 (WHSC1L), ASH1L [177] and SETMAR [178] (Figure 1). Set2/SET2 is conserved from yeast to mammals whereas NSD1-3 and ASH1L are homologous to Maternal-effect sterile 4 (Mes-4)/MES-4 and Ash1/LIN-59 in *Drosophila* and *C. elegans* [177, 179-182]. In yeast, Set2 is required for all of the mono-, di-, and trimethylation of H3K36 [183, 184]. *C. elegans*, *Drosophila* and mammalian Set2/SET2 constitute a major H3K36 trimethyltransferase [185-187] whereas NSD1-3 and their *Drosophila* ortholog Mes-4 preferentially mono- and dimethylate H3K36 [179, 188-191]. A reported loss of H3K36 trimethylation in the absence of NSD1 and Nsd2 could be explained by a requirement of mono- or dimethylated H3K36 for SET2/Set2 function [189, 192]. Among the family of SET domain-containing proteins, ASH1L is most closely related to SET2 and NSD1-3, and therefore, not surprisingly, also catalyzes H3K36 dimethylation [21, 23, 193]. Interestingly, both ASH1L and NSD1 contain an autoinhibitory loop between their SET and Post-SET domains, and at least in the case of NSD1 autoinhibition, can allosterically be relieved by interaction of the enzyme with nucleosomal DNA. Similarly, SETMAR (METNASE) regulates H3K36 dimethylation on histone octamers in vitro and DNA double-strand breaks in vivo [178, 194]. Non-histone substrates have been described for NSD1 and SETMAR (Table 1, Box 1) and different histone substrate specificities besides H3K36 have been reported for SET2, NSD1-3, and ASH1L. Other SET domain-containing proteins such as SMYD2 and SETD3 were also shown to regulate H3K36 methylation [177]. It appears that at least some of these findings are either the result of utilizing non-physiological substrates or can be attributed to indirect effects [177].

Transcriptional properties

Initial experiments showed that yeast Set2 is able to act as a transcriptional repressor of a *lacZ* reporter construct and the endogenous *GAL4* gene [177, 195]. Set2 interacts with the hyperphosphorylated C-terminus of Pol II and establishes H3K36 trimethylation along gene bodies during transcriptional elongation [177]. At the same time, recruitment of the Rpd3S histone deacetylase complex via Set2 preserves a deacetylated state to prevent spurious intragenic transcription [177]. In yeast, H3K36 methylation-dependent recruitment of the Rpd3S complex is achieved by the combinatorial requirement for two Rpd3S complex-specific subunits –Eaf3 (MRG15 in mammals) and Rco1- and their respective chromo- and PHD-domains [196, 197]. Apart from its role in keeping the coding regions of genes in a hypoacetylated state, Set2 also suppresses chaperone-mediated histone exchange to prevent the incorporation of acetylated histones on actively transcribed genes [198].

Association of MES-4 in *C. elegans* early embryos of previously active maternal genes and persistence of Pol II after loss of MES-4 suggests a role for the NSD family in transcriptional repression [199, 200]. Likewise, human NSD1 localizes to the promoter region of the *HOX* gene *MEIS1* and is required for its repression [201]. Nsd2 in conjunction with various transcription factors represses target genes such as the Nkx2-5 target *Pdgfra*[192]. However, NSD1-3 bind to the promoters of certain genes and appear to be required for transcription initiation [189, 202, 203]. They also control the H3K36 methylation state within the body and/or promoters of these genes. Furthermore, NSD3 forms a complex with the H3K4 demethylase LSD2 (AOF1) which is enriched genome-wide within the body of actively transcribed genes [113]. Similarly, NSD2 controls the H3K36 dimethylation pattern over the body of genes [191]. Thus, taken together, the NSD family seems to be involved in transcriptional repression, initiation, and elongation events.

Drosophila Ash1 and its mammalian homolog, ASH1L, are required for *Hox* gene expression [60, 204]. Based on the finding that ASH1L constitutes a H3K36 KMT and seems to associate with active genes, this suggests that it might be involved in the process of transcriptional elongation [60, 144]. Indeed, the *bithoraxoid* ncRNA in *Drosophila* recruits Ash1 in *cis* to induce transcription of *Ubx*, and a recent mammalian study implicated ASH1L in a similar event [60, 205]. Here, the ncRNA *DBE-T* drives derepression of a repeat element in muscular dystrophy patients by recruiting ASH1L. ASH1L in turn catalyzes the dimethylation of H3K36 at this locus resulting in transcriptional induction [205].

Histone H3K36 methylation and splicing

Lately, correlative evidence points towards a possible role for H3K36 methylation in splicing events. Several studies reported that nucleosomes and H3K36 trimethylation are generally more highly enriched on exons than introns [206-208]. However, confirmatory experimental evidence has largely been lagging behind. For example, SET2 has been implicated in controlling splice site switching. Exons, which are being excluded, are thought to be more highly enriched for H3K36 trimethylation, allowing recruitment of MRG15 via its chromodomain. MRG15 in turn is able to bind polypyrimidine tract-binding protein (PTB), which is known to antagonize exon inclusion, thus providing a possible explanation for exon definition in tissue-specific splicing [209]. On the other hand, the splicing mechanism itself is required for appropriate SET2 recruitment and proper H3K36 methylation suggesting a bidirectional reinforcement between SET2-dependent H3K36 methylation and the splicing machinery [210, 211].

Histone H4K20 methyltransferases

Substrate specificity

X-ray crystallographic studies of SETD8 (PR-SET7, SET8) in combination with nuclear magnetic resonance and in vitro assays on recombinant nucleosomes indicate that SETD8 is a major H4K20 monomethyltransferase [212, 213]. In mice Suv420h1 and Suv420h2 are redundantly required to implement bulk H4K20 di- and trimethylation [214, 215] (Figures 1-2). Implementation of H4K20 monomethylation by Setd8 is essential for proper H4K20 di- and trimethylation via Suv420h1/2 [216]. Therefore, not surprisingly, loss of Setd8 results in a more severe developmental phenotype than either loss of Suv420h1 or Suv420h2 alone or a combined loss of Suv420h1/2 function. Setd8 knockout leads to lethality before the embryonic eight-cell stage, and this developmental arrest depends on the catalytic activity of Setd8 [216]. *Suv420h1* knockout and *Suv420h1/2* double-knockout mice either die perinatally or develop normally as in the case of *Suv420h2* knockouts [214]. In agreement with the mammalian findings, the *Drosophila* homologs of SETD8 and SUV420H1/2 –Pr-Set7 and Hmt4-20- are H4K20 mono-and H4K20 di- and trimethyltransferases, respectively [215, 217-219]. Usually, *Drosophila pr-set7* null mutants show lethality during the late third instar larval stage [217] while *Hmt4-20* null mutants are viable into adulthood [219]. Different non-histone substrates are also direct H4K20 KMT targets (Table 1, Box 1).

Transcriptional properties

The function of SETD8 in transcription is mechanistically not very well understood. Initially thought to be involved in transcriptional repression, accumulating evidence suggests that SETD8 is also required for the activation of gene expression. A role for Setd8 in transcriptional repression is supported by the finding that SETD8/Setd8 functions in chromatin compaction [216, 220, 221] and Pr-Set7 has been shown to be a suppressor of variegation in *Drosophila*[217]. Furthermore, some categories of repressed genes are

enriched for H4K20 monomethylation and derepressed upon depletion of SETD8 [222]. For example, SETD8 controls gene repression through L3MBTL1 which is recruited to chromatin by H4K20 monomethylation [223, 224]; and another example is SET-1, the *C. elegans* ortholog of mammalian SETD8, which controls the repression of X-linked genes during dosage compensation [225]. SETD8 also regulates gene repression indirectly by monomethylating the tumor suppressor P53 on lysine 382 [226] (Table 1). L3MBTL1 can bind to monomethylated P53, and thus keep highly responsive P53 target genes repressed [227]. Repressive roles in transcription regulation for both SUV420H1 and SUV420H2 have been reported [228-230]. SUV420H1 is a suppressor of glucocorticoid receptor (GR)-mediated induction of gene expression via its interaction with GRIP1, a coregulator of the GR pathway [229], and is involved in repression of the fetal γ globin gene [230]. However, it has been proposed that the implementation of H4K20 trimethylation through SUV420H2 represses the promoter escape of Pol II by preventing recruitment of the histone acetyltransferase MOF [228]. Interestingly, SUV420H1 exists in at least two isoforms which significantly differ in their localization patterns. Isoform1 of SUV420H1 and SUV420H2 are confined to pericentric chromatin, while isoform 2 displays a more widespread distribution pattern [231]. Therefore, it is conceivable that isoform 2 could represent a more general regulator of gene repression than isoform 1. On the other hand, H4K20 monomethylation has been reported to be highly enriched over actively transcribed genes [168, 232]; and SETD8 is involved in the activation of Wnt target genes through its interaction with lymphoid enhancing factor-1 (LEF1)/TCF4 [233]. SETD8 is also required for the regulation of TWIST1 target genes during the epithelial-mesenchymal transition (EMT) [234]. However, in this context, a dual role for SETD8 in transcriptional repression and activation for different EMT genes has been proposed confirming the above-mentioned dichotomy in SETD8 function [234].

Regulation of H4K20 KMTs

SETD8 (and with it H4K20 monomethylation) is strictly controlled during the cell cycle with the highest amounts of SETD8 in G2/M and early G1, while there are undetectable levels in the S phase [216, 235, 236]. Various enzymes regulate Setd8 stability posttranslationally at different points of the cell cycle. Degradation of SETD8 by the E3 ubiquitin ligases SCF/SKP2 [237] and $CRL4^{Cdt2}$[235, 236, 238] might provide a rationale for the low levels of SETD8 during the late G1 and S phase, although only $CRL4^{Cdt2}$ was shown to directly ubiquitylate SETD8. Despite its high levels during mitosis, SETD8appears to be turned over by APC^{Cdh1}, another E3 ubiquitin ligase, after being “primed” by a phosphorylation/dephosphorylation switch mediated by CDK1/CCND1 and CDC14 [239].

Concluding Remarks

The involvement of SET domain-containing proteins in many diverse mechanisms such as transcriptional regulation, enhancer function, mRNA splicing, DNA replication, and the DNA damage response (Box 4) either by means of methylating histones or targeting non-histone substrates (Tables 1-3, Boxes 1-2) highlights the importance of this protein family in maintaining proper tissue homeostasis. Therefore not surprisingly, the misregulation of certain histone methylation marks and the misregulation and mutation of various SET domain-containing proteins are increasingly correlated with various forms of cancer and other diseases [240, 241]. For example, the important role of MLL1 translocations in acute myeloid and lymphoid leukemia depends on the methyltransferase activity of an intact MLL1 on the sister chromosome [242]. However, for most other SET domain-containing proteins, it is currently unknown whether the SET domain plays a disease-relevant role, and if yes, whether the pathological effects are mediated via the methylation of histones or non-

histone substrates. Future research will need to address some of these questions in more detail.

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Box 1

Non-histone substrates of H3K4, H3K9, H3K27, H3K36 and H4K20 methyltransferases (KMTs)

H3K4 KMTs (Table 1)

In *Set1*, *Rad6*, and *Bre1* mutants, Dam1 methylation on lysine 233 is strongly reduced affecting proper chromosome segregation in yeast. Dam1 methylation negatively affects its phosphorylation by Ipl kinase on neighboring serines. Thus, Rad6/Bre1-mediated H2B ubiquitination appears to provide a platform on kinetochores for Set1-mediated methylation of Dam1 [243, 244].

H3K9 KMTs (Table 1)

SUV39H1 methylates CBX4 (PC2) on lysine 191. This methylation event is required for the binding of CBX4 to the ncRNA TUG1, which then targets methylated CBX4 to Polycomb bodies to repress transcription [245]. Under hypoxic conditions, G9A methylates the chromatin remodeler RUVBL2 (REPTIN) on lysine 67. Methylated RUVBL2 in turn binds to the promoters of hypoxia-responsive genes and negatively affects their expression allowing a balanced response to HIF1α-induced gene expression [246]. G9A/GLP also methylate P53 on lysine 373 [247]; WIZ on lysine 305; CDYL on lysine 135; ACIN1 on lysine 654 [248]; and C/EBPβ [249] and SETDB1 have been reported to target the HIV-1 protein Tat [250].

H3K27 KMTs (Table 1)

EZH2 directly methylates the transcription factor GATA4, the orphan nuclear receptor RORA and STAT3. GATA4 methylation on lysine 299 disrupts its interaction with the acetyltransferase p300 causing attenuated transcriptional activity of GATA4 targets [251]. Methylation of RORA on lysine 38 provides a binding platform for the DCAF1/DDB1/CUL4 ubiquitin ligase complex to dynamically control protein stability [252] and EZH2-mediated methylation of STAT3 on lysine 180 results in increased STAT3 phosphorylation and enhanced STAT3 activity in glioblastoma stem-like cells [253]. A catalytically active cytoplasmic version of PRC2 has also been reported to be involved in actin polymerization via VAV1 [254]. Currently, it is unknown whether this role of PRC2 involves its catalytic activity and the methylation of non-histone substrates in the cytoplasm.

H3K36 KMTs (Table 1)

NSD1 methylates lysines 218 and 221 of P65, a component of the NF-κB complex [255] and SETMAR automethylation mediates decreased Topoisomerase IIα chromosome decatenation [256, 257].

H4K20 KMTs (Table 1)

SETD8 directly methylates P53 on lysine 382 which promotes L3MBTl1-P53 interaction to repress P53-dependent transcription [226, 227]. Methylation of NUMB on lysines 158 and 163 by SETD8 decreases the interaction of NUMB with P53 resulting in increased P53 degradation and reduced NUMB -mediated apoptosis [258]. SETD8 plays an important role in DNA replication. In this context, methylation of PCNA by SETD8 on lysine 248 has been shown to stabilize PCNA to allow for proper DNA replication [259].

Box 2

Other SET domain-containing proteins

Apart from the H3K4, H3K9, H3K27, H3K36 and H4K20 KMTs discussed here, many other SET domain-containing proteins exist which often are involved in important developmental processes (Figure 1). Whether their catalytic function as methyltransferases is important in this context is not always clear, often because of a lack of a reported substrate or independent experimental confirmation. Particularly, the PRDM family and also other SET domain-containing proteins have been reported to be crucial developmental regulators, but either their substrates are unknown, or in many cases as for histones, questionable [68, 69]. In Tables 2 and 3, we have summarized the known biological functions of these SET domain-containing proteins, if a methylated substrate has been described. The enzyme with the most non-histone substrates to date is SETD7. Among others, SETD7methylates the tumor suppressors P53 and RB1, nuclear hormone receptors, but also components of major signaling pathways and transcription factors (Table 2). Other SET domain-containing proteins with known substrates are yeast Rkm1-4, SETD6, SETMAR, SMYD2, and SMYD3 (Table 3).

Box 3

H3K9 KMTs, neurogenesis and the hypoxia and stress response

Derepression of neuronal genes can be observed in brains of *G9a* or *Glp* knockout mice and is accompanied by various behavioral phenotypes such as cognitive disabilities and autistic-like features. This role in neurogenesis also appears to be conserved for *Drosophila* G9a [260-262]. Furthermore, cocaine-induced vulnerability to stress has been linked to a loss of G9a function in the nucleus accumbens (NAc), an important brain reward region. Cocaine exposure results in decreased G9a/Glp levels and H3K9 dimethylation in NAc neurons by the upregulation of the transcription factor ΔFosB. Overexpression of G9a is able to reverse the depressive-like symptoms from cocaine exposure implicating G9a in this stress response pathway [263, 264]. However, G9A also represses neuronal genes in nonneuronal cells in cooperation with the Mediator and REST complexes [265, 266]. Hamlet, the *Drosophila* homolog of PRDM3/16, specifies the neuronal fate in the peripheral nervous system and plays a role in nascent olfactory receptor neurons where it opposes Notch signaling-induced transcriptional responses [89, 267]. Defects in the peripheral nervous system have also been reported for *Prdm3* mutant mice and Prdm16 expression appears to be sensitive to Notch pathway activity in the telencephalon [92, 268]. Many reports have implicated PRDM3/16 in transcriptional repression and activation via interaction with other co-repressors and co-activators, but currently it is unclear whether these also depend on the H3K9 monomethyltransferase function of PRDM3/16 [68, 69]. As PRDM3/16 have been reported to monomethylate H3K9 in the cytoplasm, the nuclear role in transcription activation and repression might be independent of the KMT activities of PRDM3/16.

Some H3K9 KMTs are also involved in cellular response mechanisms to hypoxia. For instance, transcriptional repression of the tumor suppressor RUNX3 under hypoxic conditions depends on the catalytic activity of G9A [269]. Upon hypoxic stress in the fetal lung, Suv39h1/2 localize to the promoter of the *SP-A* gene resulting in increased H3K9 di- and trimethylation counterbalancing its expression through hypoxic transcription factors [270]. Sirtuins are NAD+-dependent deacetylases that are sensors of oxidative stress. SIRT1 recruits SUV39H1 to chromatin and deacetylates the SET domain of SUV39H1 positively regulating its activity [271]. The activation of SUV39H1 can be reversed by DBC1, an inhibitor that can bind to the SET domain of SUV39H1 [272]. Additionally, SIRT1 controls the half-life of SUV39H1 by preventing its polyubiquitination through MDM2 on lysine 87. This stabilizes SUV39H1 and implies a major role for SUV39H1 in the control of oxidative and metabolic stress [273].

Box 4

KMTs, DNA replication and the DNA damage response

H3K4 KMTs

In yeast, H3K4 trimethylation marks meiotic recombination sites, and in *Set1* mutants double strand break formation (DSB) formation is strongly impaired [274]. For efficient meiotic recombination to occur, in-loop sequences in promoters must be connected with DSB proteins on chromosome axes. Set1/COMPASS achieves this by means of its Cps40 subunit. Cps40 binds to promoters via a PHD finger domain and promotes DSB formation by tethering in-loop sequences to chromosome axes [275, 276]. Interestingly, DSB repair by non-homologous end joining in yeast also depends on Set1; and Set1 and H3K4 trimethylation increase upon DNA damage on newly created DSB sites, possibly implying a more general role for Set1 and H3K4 trimethylation in DSB-mediated processes [277].

H3K36 KMTs

Set2 is vital for the process of DNA replication as loss of Set2 causes delayed loading of Cdc45 at origins of replication as supported by studies in budding and fission yeast [278-280]. Furthermore, NSD2 coordinates the DSB response through a module that includes the γH2AX-MDC1-dependent recruitment of NSD2 to H4K20-methylated sites of DSBs, which is followed by 53BP1 binding [281, 282]. Whether the effects on H4K20 methylation during DSB repair are the direct result of NSD2 function is not clear. Based on what is known about the substrate specificity of H4K20 KMTs, we propose that either SETD8 and/or SUV420H1/2 may be affected by NSD2 in this context. Likewise, SETMAR controls DSB repair by nonhomologous end-joining by catalyzing H3K36 dimethylation on DSBs to enhance recruitment of early DSB factors such as NBS1 and KU70 [178, 194].

H4K20 KMTs

SETD8 promotes the assembly of pre-replication complex components during late M and early G1 phase to allow for proper replication origin licensing, which also depends on SUV420H1/2 and H4K20 trimethylation. Interaction of SETD8 with PCNA at replication origins through a conserved motif results in degradation of SETD8 providing an explanation for the low SETD8 levels from the late G1 to S phase [283-287] (Table 1). Furthermore, SETD8 and SUV420H1/2 also constitute major regulators of the DNA damage response by providing an H4K20 monomethyl/dimethyl platform for 53BP1, and at least in the case of SETD8, recruitment upon DNA damage is achieved by PCNA [214, 216, 218, 220, 235, 236, 283].

Highlights

- An overview on the substrates of SET domain-containing proteins is provided.
- Protein complexes of SET domain-containing proteins are described.
- Biological functions of different classes of SET domain-containing proteins are discussed.

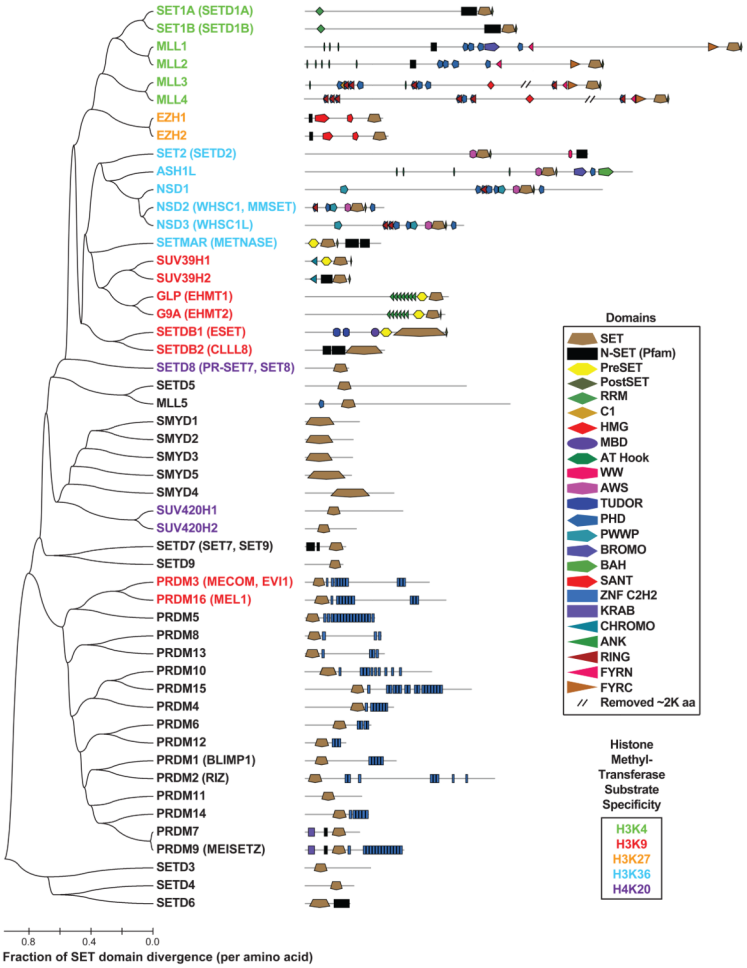

Figure 1. Relationship and structure of human SET domain-containing proteins

51 human SET domain-containing proteins were aligned according to their annotated SET domain by using ClustalO v. 1.1.0. The length of each tree branch to the next branch point constitutes a readout for the unit change per amino acid as displayed on the axis at the bottom of the tree. For each SET domain-containing protein the name(s) and corresponding domain structure are provided. The allocation and annotation of each domain structure follows SMART or NCBI (for PRDM10, SETD3, SETD4 and SETD9) as accessed on April 9, 2013. Symbols and names for depicted domains are displayed in the box labeled "Domains". Approximately 2000 amino acids (~2K aa) from the domain structure of MLL3 and MLL4 were removed as indicated by two parallel slashes. SET domain-containing proteins that show specificity towards the same histone residue (see also Figure 2) are highlighted in the same color. Colors are: green: histone H3K4 lysine methyltransferases (KMTs); red: H3K9 KMTs; orange: H3K27 KMTs; blue: H3K36 KMTs; purple: H4K20 KMTs.

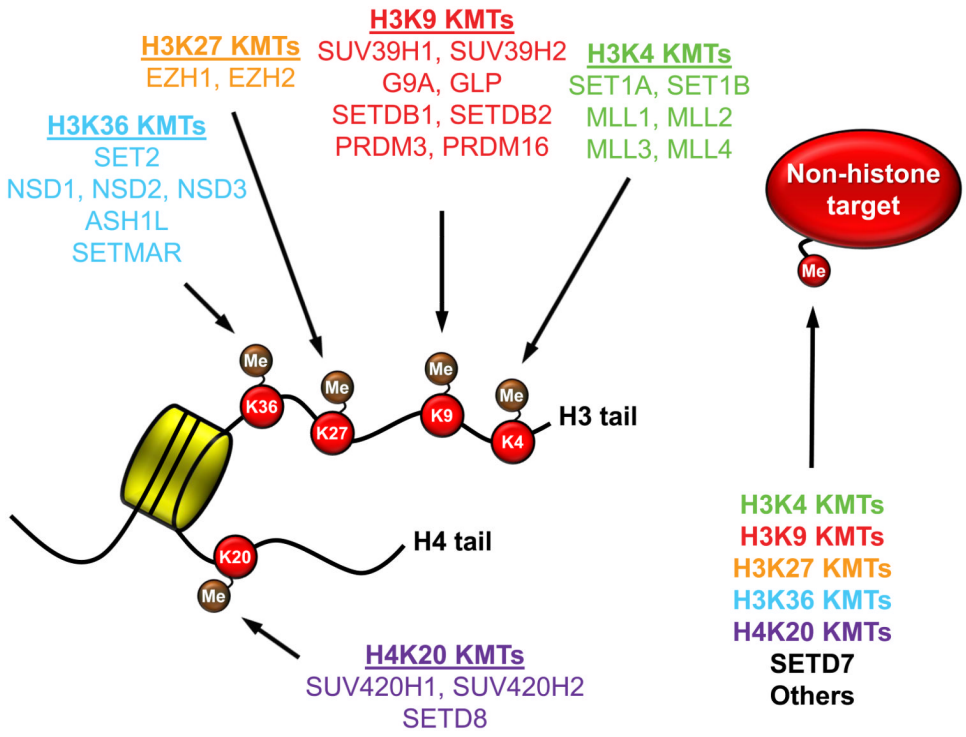

Figure 2. Histone lysine methyltransferase target specificities of mammalian SET domain-containing proteins

Green: H3K4 histone lysine methyltransferases (KMTs); red: H3K9 KMTs; orange: H3K27 KMTs; blue: H3K36 KMTs; purple: H4K20 KMTs. Non-histone substrates have been described for members of all five KMT families, SETD7 and other SET domain-containing proteins. A summary of all non-histone targets described to date can be found in Tables 1-3.

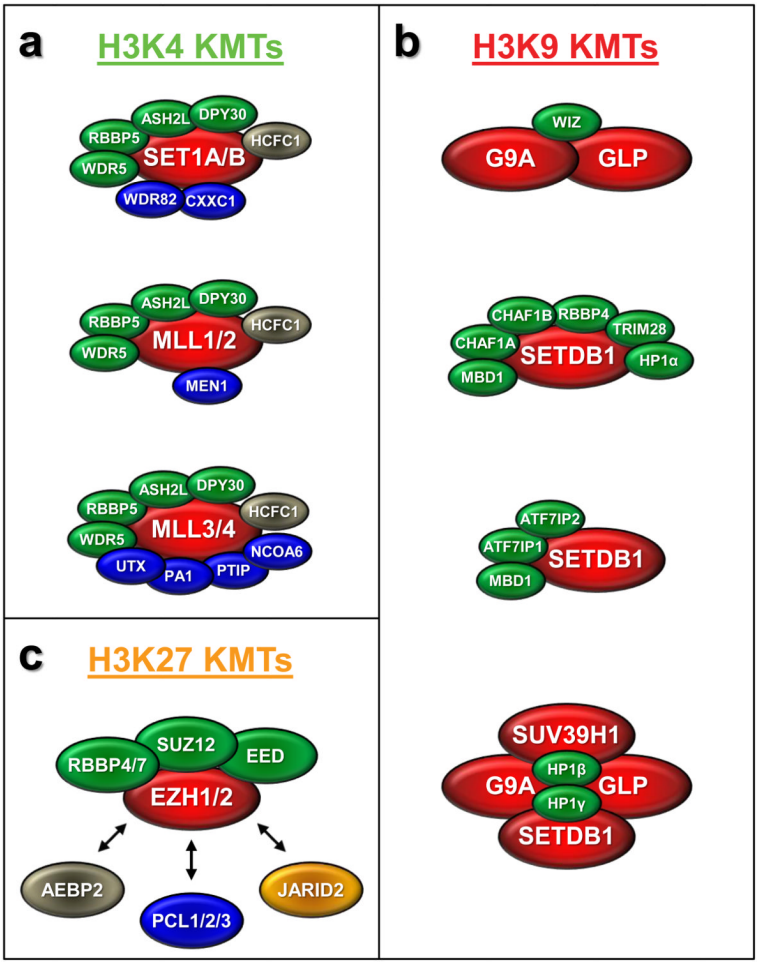

Figure 3. Mammalian protein complexes of SET domain-containing proteins described to date

All SET domain-containing proteins are highlighted in red. (*a*) Six COMPASS-like complexes for mammalian H3K4 KMTs have been described consisting of three "subbranches" (SET1A/B, MLL1/2 and MLL3/4). All complexes share core subunits which are indicated in green. Complex-specific subunits are highlighted in blue. All mammalian COMPASS-like complexes additionally contain HCFC1 highlighted in gray which is not conserved in yeast. (*b*) Four complexes for mammalian H3K9 KMTs have been reported: a heterodimeric G9A/GLP complex together with the multi-zinc finger protein WIZ; an S-phase specific SETDB1 complex with the subunits MBD1, the chromatin assembly factor subunits CHAF1 A, CHAF1B, RBBP4 and the subunits TRIM28 and HP1α; a SETDB1 complex with the subunits MBD1, ATF7IP1 and ATF7IP2; a quaternary mega complex consisting of the H3K9 KMTs G9A, GLP, SUV30H1, SETDB1 and HP1β, HP1γ (*c*) H3K27 KMT complexes consist of either EZH1 or EZH2, the core subunits RBBP4 or RBBP7, SUZ12, EED (highlighted in green) and accessory factors such as AEBP2 (gray), PCL1 or PCL2 or PCL3 (all in blue) and JARID2 (orange) that either modulate PRC2 activity and/or are required for the context-specific recruitment of PRC2 to chromatin.

Table 1

Non-histone targets of H3K4, H3K9, H3K27, H3K36 and H4K20 KMTs

KMT family	SET domain-containing protein	Substrate	Lysine(s)	Function	Reference
H3K4 KMT	Set1 (yeast)	Dam1 (yeast)	233 (me2)	Proper chromosome segregation	[243, 244]
H3K9 KMT	G9A	ACIN1	654 (me2, me3)	Unknown	[248]
H3K9 KMT	G9A	CDYL	135 (me1, me2, me3)	Decreased interaction with H3K9me3	[248]
H3K9 KMT	G9A	DNMT1	70 (me2)	Unknown	[248]
H3K9 KMT	G9A/G9a GLP/Glp	DNMT3/Dnmt3a	47 (me1, me2) (human), 44 (me1, me2) (mouse),	Binding of MPHOSPH8 to DNMT3A	[288]
H3K9 KMT	G9A	G9A	165 (me2, me3)	Required for binding of HP1α and HP1γ	[109]
H3K9 KMT	G9A	G9A	185 (me2, me3)	Unknown	[248]
H3K9 KMT	G9A GLP	P53	373 (me2)	Unknown	[247]
H3K9 KMT	G9A	RUVBL2/REPTIN	67 (me1)	Negative regulation of hypoxia-inducible genes	[246]
H3K9 KMT	G9A	WIZ	305 (me2, me3)	Unknown	[248]
H3K9 KMT	GLP	GLP	205	Binding of MPHOSPH8 to GLP	[288]
H3K9 KMT	SETDB1	Tat (HIV protein)	50, 51	Inhibition of HIV transcription	[250]
H3K9 KMT	SUV39H1	CBX4/PC2	191 (me2)	TUG1 ncRNA-dependent recruitment to Polycomb bodies	[245]
H3K27 KMT	EZH2	GATA4	299 (me1)	Decreased GATA4-dependent transcriptional activation	[251]
H3K27 KMT	EZH2	RORA	38 (me1)	Enhanced proteasomal degradation	[252]
H3K27 KMT	EZH2	STAT3	180	Increased STAT3 phosphorylation, enhanced STAT3 activity	[253]
H3K36 KMT	NSD1	RELA/P65	218 (me1), 221 (me2)	Activation of NF-κB target genes	[255]
H3K36 KMT	SETMAR	SETMAR (automethylation)	485 (me1)	Decreased Topoisomerase IIα-mediated decatenation	[256]
H4K20 KMT	SETD8/PR-SET7/SET8	NUMB	158 (me2), 163 (me2)	Decreased interaction with P53, ubiquitination-dependent P53 degradation	[258]
H4K20 KMT	SETD8/PR-SET7/SET8	P53	382 (me1)	Promotes interaction with L3MBTL1 to represses P53-dependent transcription	[226, 227]
H4K20 KMT	SETD8/PR-SET7/SET8	PCNA	248 (me1)	Stabilization of PCNA, proper DNA replication	[259]

The methylation state of the respective lysine (if known) is indicated in brackets with me1 for monomethylation, me2 for dimethylation and me3 for trimethylation. However, many reports do not necessarily exclude the possibility that other methylation states exist on the same lysine.

Table 2
Non-histone substrates of SETD7/SET7/SET9

SET domain-containing protein	Substrate	Lysine(s)	Function	Reference
SETD7/SET7/SET9	Androgen receptor (AR)	630 and/or 632 (me1)	Stimulation of AR-dependent transcription	[289, 290]
SETD7/SET7/SET9 Setd7/Set7/Set9	DNMT1/Dnmt1	142 (me1), 1094 1096 (mouse)	Enhanced proteasomal degradation	[291-293]
SETD7/SET7/SET9	E2F1	185 (me1)	Enhanced proteasomal degradation, prevention of apoptosis	[294]
SETD7/SET7/SET9	Estrogen receptor α (ERα)	302 (me1)	Stabilization of and increased transactivation by ERα	[295]
SETD7/SET7/SET9	FOXO3	271 (me1)	Decreased protein stability	[296]
SETD7/SET7/SET9	NR1H4 (FXR)	206	Enhanced binding to response elements and transactivation	[297]
SETD7/SET7/SET9 Setd7/Set7/Set9	P53	372 (me1) 369 (me1) (mouse)	Increased half-life by preventing proteasomal degradation and transactivation potential (function challenged by two new reports)	[298-302]
SETD7/SET7/SET9	PCAF	78 (me1), 89 (me1), 638 (me1)	Not known	[303]
SETD7/SET7/SET9	RB1	810 (me1)	Cell cycle arrest	[304]
SETD7/SET7/SET9	RB1	873	Interaction with HP1, transcriptional repression	[305]
SETD7/SET7/SET9	RELA/P65	37 (me1)	Enhanced promoter	[306, 307]
			binding, activation of NF-κB target genes	
SETD7/SET7/SET9	RELA/P65	314 (me1), 315 (me1)	Enhanced proteasomal degradation, repression of NF-κB target genes	[308, 309]
SETD7/SET7/SET9	SIRT1	Multiple lysines	Unknown	[310]
SETD7/SET7/SET9	STAT3	140 (me2)	Downregulation of STAT3-dependent transcription	[311]
SETD7/SET7/SET9	TAF10	189 (me1)	Stimulation of TAF10-dependent transcription	[312]
SETD7/SET7/SET9	Tat (HIV protein)	51 (me1)	Stimulation of HIV transcription	[313]
SETD7/SET7/SET9	YAP1	494 (me1)	Cytoplasmic retention of YAP1	[314]

The methylation state of the respective lysine (if known) is indicated in brackets with me1 for monomethylation, me2 for dimethylation and me3 for trimethylation. However, many reports do not necessarily exclude the possibility that other methylation states exist on the same lysine. For a more detailed review on the function of SETD7/SET7/SET9 methylated non-histone substrates we refer to [16].

Table 3
Non-histone substrates of other SET domain-containing proteins

SET domain-containing protein	Substrate	Lysine(s)	Function	Reference
Rubisco LSMT (tobacco)	Rubisco LSMT (tobacco)	14 (me3)	Not known	[315, 316]
Rkm1 (yeast)	Rpl23ab (yeast)	105 (me2), 109 (me2)	Not known	[317, 318]
Rkm2 (yeast)	Rpl12ab (yeast)	3 (me2), 10 (me3)	Not known	[319]
Rkm3 (yeast)	Rpl42ab (yeast)	40 (me1)	Not known	[320]
Rkm4 (Set7) (yeast)	Rpl42ab (yeast)	55 (me1)	Not known	[320]
SETD6	RELA/P65	310 (me1)	Repression of NF-κB target genes via the H3K9 methyltransferase GLP	[321, 322]
SMYD2	P53	370 (me1)	Represses P53-dependent transcription	[323-326]
SMYD2	HSP90	209, 615 (me1), 616 (me1)	Myofilament organization	[327, 328]
SMYD2	RB1	810 (me1)	Stimulates E2F1-mediated transcription and cell cycle progression	[329]
SMYD2	RB1	860 (me1)	Enhances L3MBTL1 binding to RB1	[330]
SMYD3	FLT1/VEGFR1	831 (me2)	Enhanced VEGFR1 kinase activity	[27]

The methylation state of the respective lysine (if known) is indicated in brackets with me1 for monomethylation, me2 for dimethylation and me3 for trimethylation. However, many reports do not necessarily exclude the possibility that other methylation states exist on the same lysine.