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Set2 mediated H3 lysine 36 methylation: Regulation of transcription elongation and implications in organismal development

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Abstract

Set2 is a RNA Polymerase II (RNAPII) associated histone methyltransferase involved in the co-transcriptional methylation of the H3 K36 residue (H3K36me). It is responsible for multiple degrees of methylation (mono-, di-, tri-), each of which has a distinct functional consequence. The extent of methylation and its genomic distribution is determined by different factors that co-ordinate to achieve a functional outcome. In yeast, the Set2-mediated H3K36me is involved in suppressing histone exchange, preventing hyperacetylation and promoting maintenance of well-spaced chromatin structure over the coding regions. In metazoans, separation of this enzymatic activity affords greater functional diversity extending beyond the control of transcription elongation to developmental gene regulation. This review focuses on the molecular aspects of the Set2 distribution and function, and discusses the role played by H3 K36 methyl mark in organismal development.

Introduction

The eukaryotic genome is packaged into a nucleoprotein complex termed chromatin, consisting of repeating units called nucleosomes. Each nucleosome consists of a proteinaceous core composed of four pairs of histone proteins, around which is wrapped ~146 basepairs of DNA. While this arrangement is necessary to accommodate the genome within the confines of the nucleus, it prevents access to the DNA for processes such as transcription and replication. Studies on individual gene loci have shown that positioned nucleosomes at promoters are unfavorable to initiation of transcription. Therefore, transcription initiation requires the disassembly or mobilization of the nucleosome[1], followed by post-transcriptional reassembly or replacement of the nucleosome. The cell has evolved specific mechanisms, allowing the transcription machinery access to DNA. This includes histone modifications, histone chaperones and chromatin remodeling complexes, the combined action of which regulates nucleosome stability. In chromatin-based regulation of transcription, histone modifications play a central role as they are used as a signal or a dock for effector proteins modulating downstream events, for as long as they persist.

Several proteins involved in the process of transcription elongation are also known to be necessary for chromatin maintenance during transcription. A key histone modification in this process is the co-transcriptional methylation of H3K36me by Set2. We review the roles of Set2 in the establishment and maintenance of the H3 K36 methyl mark, and its functional consequences to achieve complex and critical outcomes such as organismal development.

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Set2-mediated H3 K36 methylation

Transcription over a gene proceeds through three distinct stages of initiation, elongation and termination, with each stage requiring a defined set of factors that modulate each process[2, 3]. Initiation of transcription on a chromatin template begins with nucleosomal destabilization over the promoter. Nucleosomal depletion over the promoters occurs through a combination of different processes including histone modifications, chromatin remodeling and the presence of DNA-sequences influencing nucleosome stability. These events promote the binding of DNA sequence-specific activator proteins that recruit the RNAPII and general transcription factors to the promoter.

One of the key features of the transcription cycle is the phased manner in which several different proteins are recruited to the gene to aid RNAPII. These dynamic associations coordinate the different events of transcription to achieve proper gene expression. What facilitates these associations is an unusual structural feature of the RNAPII enzyme. The C-terminal domain (CTD) of the largest subunit of RNAPII, Rpb1 consists of tandem heptapeptide repeats (Y_1-S_2-P_3-T_4-S_5-P_6-S_7) that are highly conserved across species[4,5]. This domain forms a platform through which different proteins interact with RNAPII. The dynamism of these interactions is facilitated by the sequential, reversible phosphorylation of S_5 and S_2 residues of the CTD repeats in a transcription-stage specific manner (Fig. 1). Upon formation of the preinitiation complex (PIC), the kinase activity of TFIIH (Kin28 in yeast and Cdk7 in higher eukaryotes) phosphorylates the S_5 residue. This activity recruits different transcription elongation complexes including Set1C, Paf1C, Spt6 (independent of CTD[6])and FACT[7] (Spt16, Pob3, Nhp6a and b), allowing the RNAPII to proceed into elongation. About 500–750 base pairs (bp) into the gene body, Rtr1, a phosphatase associated with the RNAPII and Ssu72, dephosphorylates the S_5 residue[8, 9], resulting in the gradual loss of S_5 phosphorylation mark towards the 3′ ends of genes. After the RNAPII clears the promoter, the C-terminal domain kinase-1 (CTDK-1)(catalytic subunit Ctk1 in yeast and Cdk9 in higher organisms), phosphorylates the S_2 residue, stimulating the recruitment of the Set2 histone lysine methyltransferase[10] (KMT). This KMT catalyzes the transfer of multiple methyl groups (mono-, di-, tri-methylation) on the histone H3 K36 residue. The various degrees of methylation are distributed in a graded manner over the coding region (Fig. 1, bottom) with H3 K36 mono-methylation (H3K36me1) over the 5′ ends, di-methylation (H3K36me2) over mid-coding regions, and tri-methylation (H3K36me3) towards the 3′ ends of genes[11]. This distribution is determined by the association of different regulatory proteins with Set2 (as discussed in the next section) and the transcription status of the gene[12] ultimately affecting its function.

The Set2 protein and its KMT activity are conserved across species. However, Set2 orthologs in metazoans (Table 1) have evolved to include several structural and functional features that distinguish them from the yeast counterpart. They show considerable variation in the degree of methylation catalyzed as well as the substrates targeted. One striking feature is the separation of the H3 K36 mono-/di-methylation activity from the tri-methylation activity on different proteins. This alludes to the distinct functions of each methyl mark, and separation of the catalytic activities is necessary for regulation based on their protein interaction profile or tissue-specific expression patterns.

Factors affecting H3K36 methylation by Set2

Set2-mediated H3K36me is modulated by a variety of factors that either associate with the enzyme or belong in the same pathway (Table 2). In this section, we outline the different chromatin related factors that influence the activity of Set2 and thereby the degree and distribution of the H3 K36 methyl mark.

Domains within the Set2 protein regulate its lysine methylation activity (Fig. 2). The evolutionarily conserved catalytic site is found within a ~130 amino-acid (a.a) SET domain, named after three *Drosophila* genes containing this domain, Su(var)3–9, E(z) and trithorax[13]. This region is flanked on both sides by cysteine-rich regions, the Associated with SET (AWS) and the Post-SET domains that are required for the methyltransferase activity[14]. These domains are thought to determine the specificity of the lysine residue targeted for methylation[15, 16]. The active site in the SET domain accommodates the aliphatic side chain and the ε-amino group of the lysine residue and requires a methyl donor, S-adenosyl-L-methionine (AdoMet) as a cofactor. The structure of SET domain allows the substrate and cofactor separate access to the catalytic site such that the cofactor can transfer the methyl group to the active site and leave[17]. This feature is useful for the SET domain containing proteins to allow multiple degrees of methylation without the substrate having to leave the active site. Interestingly, the presence of a phenylalanine or tyrosine at the catalytic site of SET domains determines the degree to which the target lysine can be methylated. For example mutating F281 to Y in the *N. crassa* Dim-5 methyltransferase, converts it from an H3K9 trimethylase to a mono-/dimethylase[18]. Thus, stearic hindrance in the active site is responsible for limiting the degree to which an enzyme can methylate lysine substrates[19].

Set2 can methylate both histone octamers and nucleosomes[20]. Studies with yeast Set2 revealed that histone H4 allosterically activates H3K36me. Further analysis led to the identification of a *trans*-histone methylation pathway with the H4 K44 residue necessary for positioning the active site of Set2 on H3 K36 on nucleosome templates[14]. Additionally, residues on H2A and H3 surrounding H4 K44 in nucleosome are also required for H3K36me[21]. Set2 interacts with this region through a nine-residue domain at its N-terminus (Fig. 2). Loss of this domain reduced Set2 binding to nucleosomes and abrogated di- and tri-methylation of K36, while binding the CTD was unaffected[14]. While Set2 binds directly to unmodified H3 K36, initiating catalysis, the addition of methyl groups inhibits direct binding. Therefore, the *trans*-histone methylation pathway provides an anchor for the Set2 enzyme thereby facilitating multiple methylation of H3 K36. Interestingly, the human nuclear receptor SET domain-containing-2 (NSD2) enzyme that catalyzes H3 K36me1 and H3K36me2, also targets H4 K44 when methylating histone octamers but not nucleosomes. However, while H3K36me by NSD2 requires H4 K44, it is independent of its methylation status[22].

The interaction of Set2 with the phosphorylated CTD of RNAPII targets it from the middle to 3′ end of genes. This association with the S_5 and S_2 di-phosphorylated CTD occurs through its C-terminal Set2-Rpb1 interaction (SRI) domain[23]. Optimal binding of SRI to the CTD requires a minimum of two S_5/S_2 di-phosphorylated heptad repeats[24, 25]. Loss of the SRI domain abolished di- and tri-methylation of H3 K36 (Fig. 2), although it had no effect on mono-methylation[14]. Further deletion of the C-terminal domain of Set2 can partially restore di-methylation[26]. Loss of the CTDK-1 kinase subunit, Ctk1 abrogates methylation of H3 K36[27, 28], while deletion of *BUR1* (another Cdk9 ortholog in yeast) results in the loss of H3 K36 tri-methylation only[29]. The Bur1-Bur2 cyclin-dependent kinase is required for the recruitment of the Paf1 complex and subsequent H2B ubiquitylation and H3 K4 methylation[30–32]. Loss of either Paf1 or its interacting partner Ctr9, results in loss of H3 K36 tri-methylation[33] presumably by affecting the transcription elongation pathway. Interestingly, all these mutations also perturb the S_2 phosphorylation levels on RNAPII CTD, resulting in destabilization of the Set2 protein[34].

The requirement of amino acid residues on H4, H2A and H3 for H3K36me indicates that Set2 needs intact nucleosomes to methylate its target lysine, *in vivo*. Consistent with this observation, a number of histone chaperones involved in maintenance of nucleosomal structure during elongation are essential for H3K36me3. In humans, the recruitment of the

H3 K36 tri-methyltransferase SETD2 has been dissected with respect to Spt6, which is the histone chaperone involved in post-elongation nucleosomal reassembly. It is a phospho-S_2 binding protein[26, 35] that targets the proximal conserved heptad repeats. Spt6-CTD binding is necessary for the recruitment of Interacts with Spt6-1 (Iws1), which in turn recruits the SETD2 protein[36]. These observations suggest that the tri-methylation of H3 K36 is coupled with reassembly of the nucleosome after the passage of RNAPII.

Both the FACT complex[29] and Asf1[37], histone chaperones involved in transcription elongation[38, 39], are also necessary for maintaining H3 K36 tri-methylation but loss of either factor does not affect mono- or di-methylation. The FACT complex has been shown to facilitate the reassembly of H2A-H2B dimers that are preferentially mono-ubiquitinated on histone H2B[40, 41]. Consistent with this fact, loss of Large cells 1 (Lge1) a component of the yeast Bre1-ubiquitin ligase complex is associated with a loss of H3 K36 tri-methylation only[42].

Other factors that influence H3 K36 tri-methylation include the Fpr4 prolyl isomerase and the RNA-associated protein HnRNPL. Fpr4 mediated prolyl isomerization of the H3 P38 residue renders the conformation of the H3 K36 residue unsuitable for tri-methylation[43]. HnRNPL associates with the human SETD2 protein and promotes the trimethylation of the target lysine[44]. Interestingly, HnRNPL has been implicated in regulating splicing, which in turn has been shown to activate H3 K36 tri-methylation[45].

In addition to the factors that interact with Set2 and modulate its activity, histone lysine demethylases (KDM) can enzymatically remove H3 K36 methyl groups. Yeast H3 K36 demethylases include Jhd1 and Rph1. Both proteins contain the Jumonji C (JmjC) domain and catalyze the oxidative demethylation of methyl-lysine targets[46]. Rph1 demethylates both H3 K36 tri- and di- methyl substrates[47, 48] while Jhd1 targets di- and mono-methyl H3 K36[49, 50]. Interestingly, metazoan H3 K36 demethylases bind H3 K4 methylated residues and are targeted to the 5′ ends of genes. This feature may be important to maintain the genomic distribution of the different H3 K36 methylated states (mono-, di-, tri-)[51, 52].

Each factor affecting H3 K36 methylation modulates the number of methyl group added. Such a level of cellular control alludes to the functional exclusivity of each degree of methylation. Therefore, the interplay between these factors over chromatin determines the degree of methylation of the target residue as well as its genomic location, creating a variety of functional outcomes. Additionally, human SET and MYND domain-containg protein 2 (SMYD2) has also been shown to methylate p53 and retinoblastoma (Rb) proteins, enhancing the functional diversity of the SET proteins[53].

Functional consequences of H3 K36 methylation

Regulation of chromatin processes by histone modifications occurs through several chromatin modifying proteins. Histone modifications can be viewed as signals on histones that are recognized by specific domains on these effector molecules. Methyl-lysine residues are recognized by a number of conserved domains including chromo-, Plant homeo domain (PHD), WD40, Tudor and PWWP domains[54]. These domains are found in a diverse range of protein complexes affecting chromatin structure, all of which coordinate to achieve specific functional consequences.

Set2/Rpd3S pathway

H3 K36 methylation is a co-transcriptional event that is enriched over the 3′ end of the coding region. Loss of Set2-mediated H3 K36 methylation resulted in hyperacetylation of histones over this region. A similar phenotype was observed on the loss of Eaf3 and Rco1,

two components of the Rpd3S histone deacetylase complex[55, 56]. The Rpd3S complex is recruited to the coding region through its interaction with the S_5 and S_2 di-phosphorylated CTD of RNAPII[57, 58]. Eaf3 is a subunit shared with the NuA4 complex[59] and contains a chromodomain that binds to methylated H3 K36 and H3 K4[55, 60]. Interestingly, the PHD domain of Rco1 mediates the modification-independent nucleosomal binding of the Rpd3S complex so as to enhance the specificity of Eaf3 towards di- or tri-methylated H3 K36[42, 61] and the subsequent deacetylation of histones H3 and H4. Thus, the Set2-mediated H3 K36 methylation coordinates the recruitment and activation of the Rpd3S complex over the coding regions, resulting in the maintenance of a hypo-acetylated state (Fig. 3). Although the H3 K36 methyl mark is present on all active genes, the Set2/Rpd3S pathway affects infrequently transcribed genes and long genes in yeast[62]. Mammalian cells also possess a complex similar to yeast Rpd3S that is involved in the deacetylation over transcribed loci[63]. Linking the co-transcriptional H3 K36 methyl mark to the activation of histone deacetylase complex ensures that the coding regions are hypo-acetylated after the passage of RNAPII.

H3K36 methylation and nucleosomal dynamics

Nucleosomal dynamics during transcription elongation is usually a consequence of the passage of RNAPII[64]. Recent *in vitro* work has shown that moderate RNAPII elongation occurs over the nucleosome with the loss of a single H2A-H2B dimer, leaving a hexameric nucleosomal complex behind[65]. In conjunction with this observation, *in vivo* studies have shown a continuous exchange of the H2A-H2B dimers over the coding regions[66]. In yeast, H3 turnover over coding regions is very slow for a majority of genes with the exception of highly transcribed genes[67, 68]. We have recently shown that the H3 K36 methyl mark over the coding region prevents the replacement of histone H3 over the coding region with newly synthesized ones[69]. Interestingly, this feature also prevents the enrichment of pre-acetylated histones over the coding region, suggesting that co-transcriptional acetylation was partly a consequence of histone exchange[69], apart from the recruitment of histone acetyltransferase complexes[70]. Indeed, targeting SET domain proteins to the promoters results in transcriptional repression[20]. Wolf-Hirschhorn syndrome candidate 1(WHSC1, formerly known as NSD2) is recruited by the cardiac-specific transcription factor Nkx2-5 and represses the platelet-derived growth factor α gene[71]. This observation can be attributed to the ability of H3K36me to suppress histone exchange and thereby promoter acetylation. This suggests that histone exchange over promoters could be involved in the maintenance of histone acetylation. In a recent study, depletion of one copy of H3 results in the loss of nucleosomes over the promoter regions while the coding regions were enriched for H3 K36 methylated nucleosomes[72], suggesting that this histone mark is required for nucleosomal retention during transcription elongation.

The suppression of histone exchange over the coding regions is achieved by H3 K36 methylation through two distinct mechanisms. H3 K36 di- and tri-methylation impedes the interaction of Asf1– a histone chaperone involved in histone exchange during transcription elongation– to histones over the coding regions[69]. At the same time, this methyl mark recruits the imitation switch 1b (Isw1b) chromatin-remodeling complex over the coding regions, which acts along with chromodomain-helicase-DNA binding-1 (Chd1) to re-establish chromatin integrity following the passage of RNAPII[73–75] (Fig. 3). These mechanisms ensure that while H2A-H2B dimer exchange occurs unimpeded, the H3-H4 tetramer core remains intact. This results in the persistence of the H3 K36 methyl mark over the coding region following transcription elongation. Recently, the common core of Rpd3S and the promoter-specific Rpd3L deacetylase complexes composed of three subunits– Rpd3, Sin3 and Ume1[55]–was shown to possess a histone modification-independent histone chaperone activity[76]. The core subunits prevent nucleosome eviction but not the remodeling activity of the RSC complex, indicating the possible involvement of another H3K36me-

recruited complex in the maintenance of chromatin integrity. Histone exchange in metazoans accompanies transcription, resulting in the replacement of the replication-specific histone H3.1 with the transcription-specific H3.3[77, 78]. Interestingly, the latter histone variant is enriched for the H3 K36 methyl mark, added following the process of transcription elongation and histone replacement[79]. The H3K36me on H3.3 may prevent it from undergoing further exchange, while facilitating further rounds of transcription.

Set2/Rpd3S pathway and cryptic transcript initiation

Transcription initiation at sites other than the 5′ end of the gene is a widespread phenomenon in *Drosophila* and humans[80]. The use of multiple start sites coupled with antisense and intergenic transcription indicates the complexity of the transcriptome. Most of these transcripts are tightly regulated, alluding to their function in critical cellular events. In yeast, loss of the Set2/Rpd3S pathway results in the widespread initiation of transcription from within the coding regions (Fig. 4), resulting in both sense and antisense transcripts[62]. Several chromatin-associated proteins regulate cryptic transcript initiation, suggesting the importance of chromatin structure in suppressing this phenotype[81, 82]. Interesting, many of these proteins are involved in the maintenance of H3 K36 methylation. Recent studies have shown that cryptic transcription is activated in response to cellular stress, and that some of these transcripts are translated, although no function has been assigned to these proteins[81].

H3 K36 tri-methylation and dosage compensation

Dosage compensation is a process that ensures gene expression is normalized between the autosomes and the sex chromosome[83]. In male flies it involves an ~ 2-fold increase in transcription from the single X chromosomes to equal that from diploid autosomes. The Male Specific Lethal (MSL) complex in male flies is targeted to the X chromosomes in a DNA sequence specific manner, followed by their spread over the chromosomes. This ectopic spreading is facilitated by H3 K36 tri-methylation by recruiting the chromodomain-containing MSL3 subunit of the MSL complex[84] to the 3′ ends of genes. The males absent on the first (MOF) subunit of the MSL complex catalyzes the acetylation of H4 K16 residue, which is involved in the maintenance of active chromatin by preventing compaction of chromatin[85] (Fig. 5a). The exact role of the MSL complex in upregulating transcription is yet to be deciphered. Interestingly, the loss of *Drosophila* Set2 results in an increase in H4 K16 acetylation over the coding regions[86].

H3 K36 methylation and Polycomb silencing

Polycomb group (PcG)-mediated silencing is involved in dosage compensation through X-inactivation, the developmental regulation of homeotic (Hox) genes and cancers[87, 88]. Two main PcG complexes have been identified that work in conjunction with each other, although some exceptions exist. Polycomb repressive complex 2 (PRC2) complex contains a histone methyltransferase targeting the H3 K27 residue. This methylation is thought to recruit the PRC1 complex resulting in silencing of the locus. PRC2 itself recognizes H3 K27 methylation, activating the methyltransferase allosterically, resulting in the spreading of the mark, and creating a silenced genomic region. Active marks, predominantly H3 K4 and K36 methylation, restrict this spreading activity of PRC2[89]. H3 K36 di- and tri-methylation prevent PRC2 complex binding and its activity[89, 90] (Fig. 5b). It has been recently shown that the PRC2 enzyme is strongly activated by dense nucleosome packing. Interestingly, amino acids 35–42 on histone H3 of the neighboring nucleosome bound an acidic region of the Su(z)12 subunit of PRC2 through electrostatic interactions[91]. Knockdown of the human SETD2 protein results in global up-regulation of H3 K27 tri-methylation levels[36]. Interestingly, in Drosophila, the antagonistic trithorax group protein Absent, small or homeotic-1-like (ASH1L) has been shown to be a H3 K36 di-methylase[90] *in vitro*. While H3K36me inhibits PRC2-mediated H3 K27 methylation, *Drosophila* Setd2 has been shown

to act on H3 K27 methylated templates, suggesting that Polycomb-mediated silencing can be reversed by active transcription.

H3K36 methylation and Alternative splicing

Alternative splicing (AS) of precursor mRNA is a co-transcriptional process, in which differentially selected splice sites result in the production of functionally distinct mRNA and protein isoforms[92]. AS is a tightly regulated process requiring the recruitment of a vast array of proteins that catalyze the splicing event. A key event in this coordinated process involves the recognition of the splice site by proteins that either promote or suppress splicing, which results in inclusion or exclusion of the exon, respectively. Polypyrimidine-tract binding protein 1 (PTBP1) protein is a splice junction-binding protein that suppresses splicing resulting in exon exclusion (Fig. 5c). PTBP1 is recruited on nascent RNA by MORF4 related gene on chromosome 15 (MRG15, a homolog of yeast Eaf3) bound to methylated H3 K36[93]. The selection of fibroblast growth factor receptor 2 (FGFR2) exon IIIc over exon IIIb in mesenchymal cells is due to the distribution of H3 K36 tri-methylation over exon IIIb[93], resulting in PTBP1-mediated exon exclusion. However, in epithelial cells, exon IIIb is included due to the epithelial splicing regulatory protein 1 (ESRP1)[94] and is not enriched for the H3 K36 methyl mark. Indeed, the epithelial-to-mesenchymal transition (EMT), a hallmark of metastasis, involves a switch from FGFR2-IIIb to FGFR2-IIIc[94], indicating a role of tissue-specific H3K36me distribution in carcinogenesis. Similarly, mouse PC4 and SF2 interacting protein 1 (Psip1) binds H3 K36 tri-methylated nucleosomes directly through a PWWP domain and regulates AS, usually at downstream exons[95]. In agreement with a role in regulating exon selection, H3K36me3 is differentially distributed between included exons and introns[96]. These observations point to the role of H3 K36 methylation in alternate splicing. The chromatin remodeler Chd1, involved in the Set2-dependent maintenance of chromatin integrity after RNAPII elongation[73], also plays a role in alternate splicing[97].

H3K36 methylation and Development

The sequential activation and regulation of gene networks is a hallmark of developmental processes resulting in morphological outcomes. Developmental regulation serves to generate a spatio-temporal pattern of gene expression that not only specifies the exact time, but also the cell in which the gene must be expressed. One point to note here is that these networks not only regulate the activation but also coordinate the repression of genes to specify cell fates. Additional regulation at RNA and protein levels, determines the precise outcome. Any perturbation in this network would lead to developmental defects and/or carcinogenesis[98]. In this section, we enumerate the developmental effects of the H3 K36 methylation pathway.

MES-4 and epigenetic germline maintenance

Divergent somatic and germline lineages are established during early division cycles of metazoan embryonic development and are maintained throughout organismal development[99]. The germ cell lineage is characterized by unique chromatin architecture, and the factors involved in the maintenance of this lineage are mostly chromatin-associated proteins. Maintenance of germ cell lineage requires a balance between gene expression and overall repression to ensure genomic integrity[99]. In particular, two classes of genes involved in chromatin state specification in *C. elegans* highlight this disparity. The maternal-effect sterile (MES) genes are maternally supplied factors that are crucial for the development of the germ cell lineage. Loss of any one of the four MES factors results in the deterioration of the primordial germ cells in the zygote, leading to the production of sterile worms[100]. MES-2, MES-3 and MES-6 form a repressive complex similar to the PRC2 complexes in other metazoans species, and are involved in silencing the X-chromosomes during germ cell development[101, 102]. MES-4 is a histone methyltransferase involved in H3 K36 methylation

(Table 1). MES-4 and the H3 K36 di-methyl mark are found enriched over the autosomes, but not over the X-chromosome (except the tip of the left arm) in the germline tissue[103, 104]. This exclusion of MES-4 over the X-chromosome is thought to be due to the MES-2,-3,-6 complex. Interestingly, loss of MES-4 results in the de-silencing of the X-chromosome.

MES-4-mediated H3 K36 methylation has some notable differences compared to the yeast Set2 protein. Although MES-4 is found over the coding regions, it does not require RNAPII for its activity or localization. Indeed, the distribution of H3 K36 di-and tri-methylation over germline-specific genes is enriched towards the 5′ end rather than the 3′ end of the gene, as expected from studies in yeast[104]. MES-4 is the predominant H3 K36 methyltransferase in the germ line cells. Methyltransferase-1 (MET-1), the SETD2 homolog catalyzing the co-transcriptional H3 K36 tri-methylation is not expressed until late embryonic stages (100-cell stage) and is not involved in germline specification or maintenance. Interestingly, MET-1 mutants in association with other specific mutations alter the somatic cell lineage to give rise to the vulva, by failing to repress the epithelial growth factor (EGF) gene *lin-3* in worms[105]. Loss of Setd2 in mice resulted in severe vascular defects[106].

Loss of MES-4 results in sterility of the progeny of homozygous mutant hermaphrodite worms. This observation indicates that normal development of germ cell in the homozygous mutant worms is due to factors transferred from the heterozygous mother. Interestingly, MES-4 and the associated H3 K36 methyl mark are distributed over the germline-expressed genes in early embryos, even in the absence of transcription. This – coupled with the observation that embryonic expression of catalytically dead *mes-4* mutant does not rescue the sterile phenotype – led to the hypothesis that MES-4 mediated H3 K36 methyl mark is an epigenetic signal necessary for the proper development of primordial germ cells[104]. Inheritance of the epigenetic marks across mitosis (and meiosis) requires the nucleosomes to be stable across the cell cycle, which is not the case due to replication dependent histone turnover. DNA methylation as well as H3 K9 and H3 K27 methylation can undergo trans-generational transfer of epigenetic information. A key event in this transfer is the ability of these marks to recruit or allosterically activate the enzymes that add them. Such an activity has not been described for the Set2 homologs as yet. How the MES-4 protein recognizes and maintains genomic regions with H3 K36 methyl mark in early embryos remains to be determined.

Mammalian homologs of the MES-4 protein include the NSD family of H3 K36 methylases (Table 1). Loss of these proteins displays a wide range of developmental defects. Homozygous mutant embryos of NSD1 die during gastrulation due to excessive apoptosis[107]. Hemi-zygous mutants of WHSC1 (formerly known as NSD2) are associated with Wolf-Hirschhorn syndrome, which is characterized by craniofacial defects and growth delays[71], suggesting a critical role of this family of proteins in embryonic development. Incidentally, *Drosophila* dSet2 is involved in larval to pupal transition[108]. Additionally, H3K36 di-methylation by the human SET domain and mariner transposase fusion gene-containing protein (SETMAR), is necessary for non-homologous end joining (NHEJ)[109], thereby maintaining genomic integrity.

Flowering control in Arabidopsis

Flowering in plants requires a change in the characteristics of totipotent apical meristem (tissue consisting of undifferentiated cells) from a vegetative (generates shoots and leaves) to a floral (generates flowers) state. In plants, activation of the sexual reproduction pathway is usually in response to environmental stimuli like day length (photoperiod) or temperature changes[110]. The response to these signals, even if they are transient, induces changes in the gene expression pattern leading to floral commitment. One of the well-studied pathways in flowering control is the regulation of the MADS-box transcription factor, *FLOWERING*

LOCUS C (*FLC*), which acts as a flowering repressor[111]. Upon receiving the necessary environmental cue, the floral transition is initiated leading to the repression of *FLC*. Loss of *EARLY FLOWERING IN SHORT DAYS* (*EFS*)/*SET DOMAIN GROUP 8* (*SDG8*), the SET domain containing H3 K36 di- and tri-methylase, results in reduced *FLC* expression, causing early flowering[112]. Interestingly, loss of *SET DOMAIN GROUP 26* (*SDG26*), an H3 K36 mono-methylase, causes late flowering, presumably by alleviating repression of *FLC*[113]. Interestingly, loss of the *Arabidopsis* PAF1 complex components cause early flowering and a loss of H3 K36 di- and tri-methylation[110], in conjunction with studies in yeast. Repression of *FLC* involves the PRC2 complex-mediated silencing of chromatin[111]. Therefore, activation of *FLC* locus can be explained in terms of *SDG8*-mediated eviction of the polycomb complex.

Interestingly, *EFS* is required for anther and ovule development in *Arabidopsis*, and its loss causes severe defects in the development of the reproductive organs[114]. *EFS* is similar to *C. elegans* MES-4, which is also involved in germline maintenance and in stress response to fungal infections.[115]

Conclusions

Set2-mediated H3K36me prevents co-transcriptional acetylation by suppressing histone exchange over the coding regions. It also recruits chromatin-remodeling complexes to the coding regions to maintain chromatin structure and nucleosomal spacing. At the same time, it activates the deacetylase activity of the elongating RNAPII-specific Rpd3S complex, removing histone acetyl marks placed by histone acetyltransferases. On the basis of its distribution, H3K36me carries out these functions towards the 3′ ends of genes. Recent studies have demonstrated similar functions for the 5′-specific H3 K4 methyl mark, although its role in affecting histone exchange has not been analyzed yet[116]. Thus, both of these methyl marks coordinate to maintain chromatin integrity over the coding regions of genes.

Associating the Set2/Rpd3S pathway with the active transcription machinery ensures efficient maintenance of chromatin integrity over the coding regions. It also affords tighter regulation at the level of each transcription cycle, including co-transcriptional events like RNA splicing. Although H3K36me serves to suppress spurious initiation of transcripts and maintain the coding regions in a hypoacetylated state, it does not impede transcription. Thus, it is not a repressive mark, but a regulatory one, which maintains chromatin structure following elongation in a state that allows further rounds of transcription through it while preventing internal initiation.

By suppressing histone exchange, H3K36me ensures its persistence across different rounds of transcription, ensuring transcriptional memory with respect to its genomic position and transcriptional status. This memory is lost upon DNA replication-associated histone exchange on chromatin. Recent studies have suggested that active chromatin is partitioned as dimers, allowing for the marks to be distributed to the daughter strands in the vicinity of the original genomic locus[117]. This observation, coupled with the trans-generational maintenance of H3K36me by *C. elegans* MES-4, truly qualifies it as an epigenetic mark.

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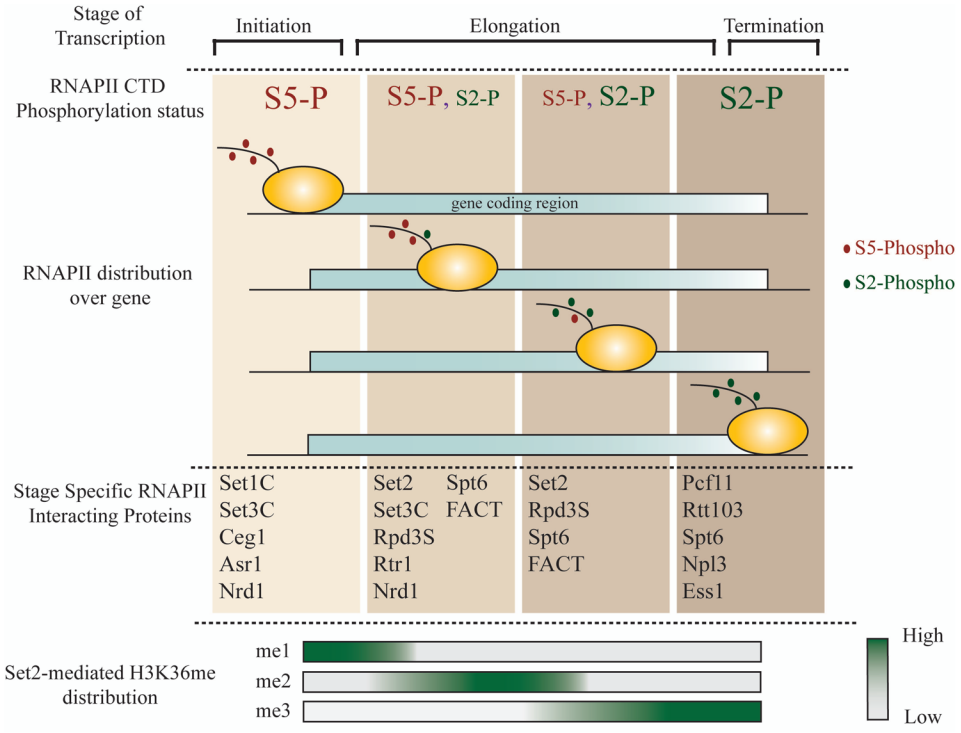

Figure 1. RNAPII CTD phosphorylation cycle across the coding region

Distribution of the RNAPII CTD phosphorylation marks across the coding region of genes. Differential phosphorylation of the S5 or S2 residues in the heptad repeat contributes to the stage-specific association of transcription regulatory proteins. The font size of the CTD phosphorylation at the top of the figure corresponds to the relative abundance of that post-translational CTD modification at a particular stage. The distribution of the Set2-dependent H3K36 methyl marks (me1, me2, me3) across the coding region is denoted as a heat map at the bottom.

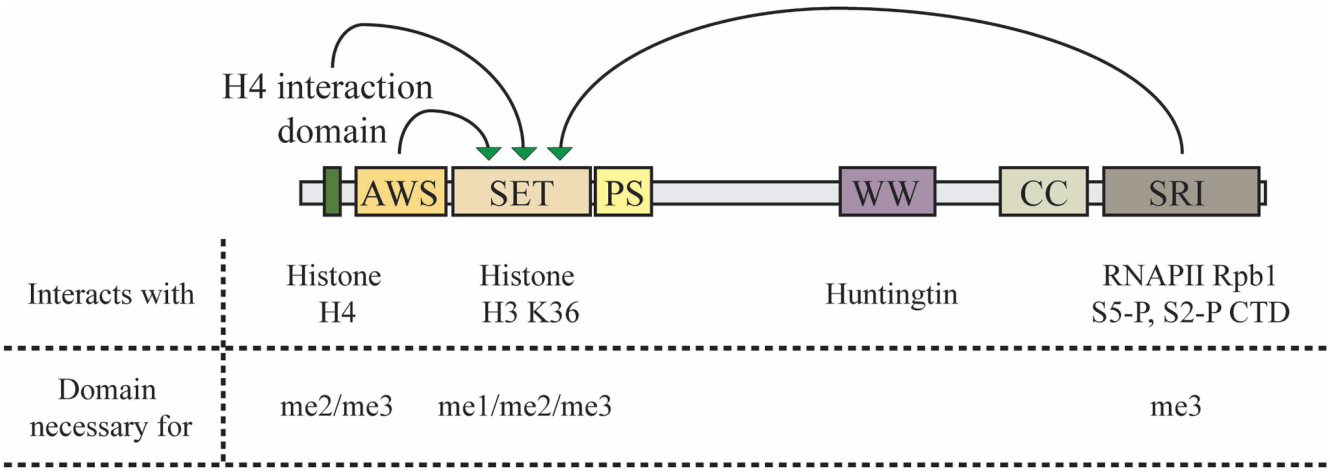

Figure 2. Set2 domain architecture and its effect on H3 K36 methylation

Domain architecture and interactions of the yeast Set2 protein and their effect on H3K36me. The green arrows indicate the necessity of the domain for the methyltransferase activity of the SET domain. AWS: Associated with SET; SET: Su(var), E(z) and Trithorax; PS: Post-SET; WW: Typtophan-rich domain; CC: coiled coil domain; SRI: Set2-Rpb1 interaction domain.

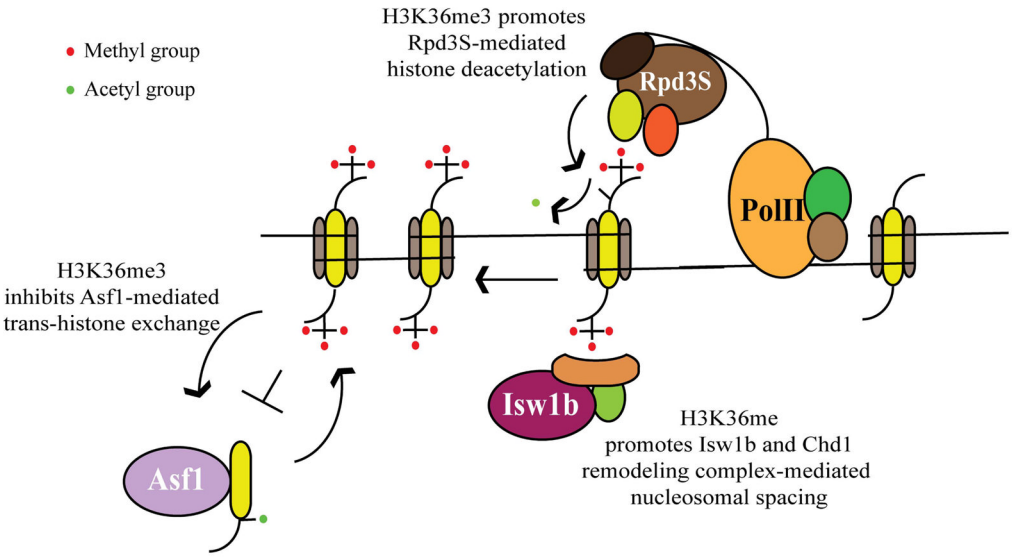

Figure 3. Role of Set2-mediated H3K36 methylation in nucleosomal dynamics

Set2-mediated K36 methylation regulates nucleosome dynamics through different mechanisms. It suppresses histone exchange over the coding regions by preventing binding of the Asf1 protein to the methylated histones, and promotes the retention of the original nucleosome. This prevents the enrichment of pre-acetylated histones on the coding regions. H3K36me also recruits the Isw1b protein complex to maintain regular nucleosomal spacing after the passage of the elongating polymerase. H3K36me also activates the deacetylase activity of the RNAPII-associated Rpd3S complex, to remove the acetyl marks that have been added by histone acetyltransferase complexes.

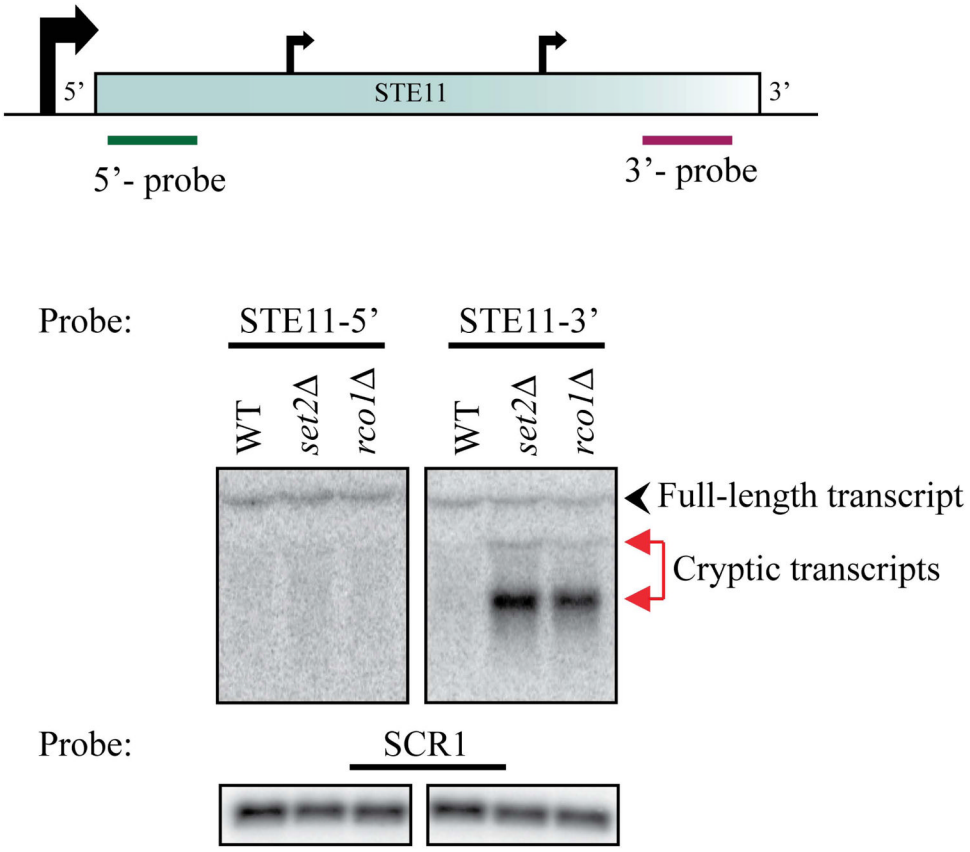

Figure 4. Cryptic transcription upon loss of Set2/Rpd3S pathway

Total RNA was prepared from the wild type, *set2Δ* and *rco1Δ* strains and subjected to Northern blot analysis. The probes used in these assays were from the 5′ and 3′ ends of the *STE11* gene (top). An SCR1 probe was used as a loading control. The full-length transcript and the short cryptic transcripts are indicated. The approximate start sites for the cryptic transcripts from within the coding regions are indicated.

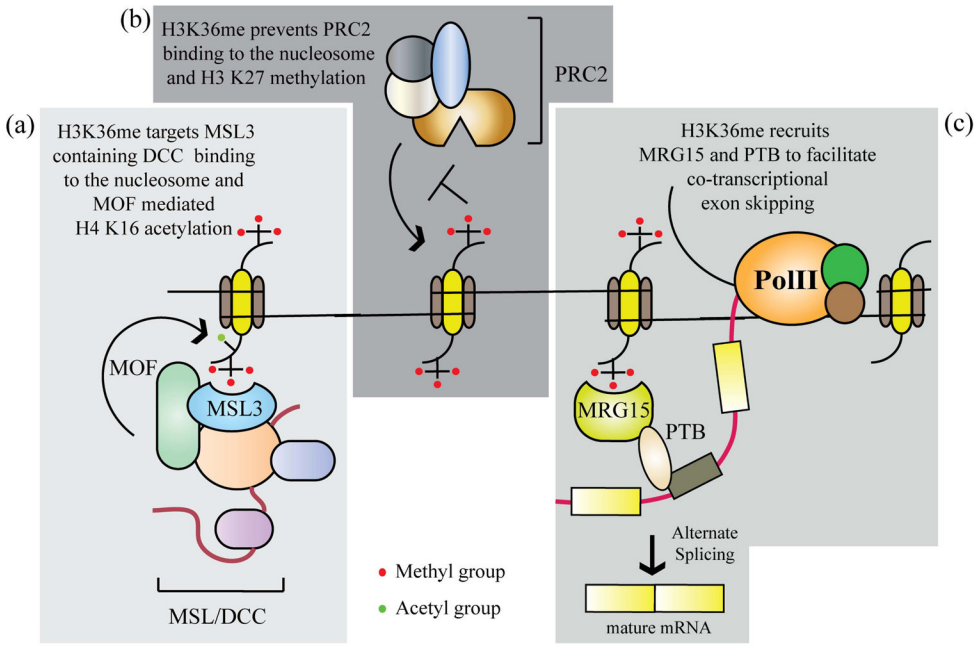

Figure 5. H3 K36 methylation in transcription regulation

H3K36me regulates transcription rates by a number of methods. (a) In dosage compensation in flies, it recruits the MSL/DCC to the coding regions of the X-chromosome, stimulating the H4 K16 acetylation by the MOF subunit. This increases the rate of transcription over the X-chromosome to equal that of the autosomes. (b) H3K36me prevents the binding and subsequent methylation of the H3K27 residue by the repressive polycomb PRC2 complexes. (c) H3K36me regulates alternate splicing of the transcript in a co-transcriptional manner. The MRG15 (yeast Eaf3) protein binds the H3K36me mark through its chromodomain. PTB binds MRG15 and facilitates exon skipping.

Table 1

Set2 Orthologs: Targets and functions

Orthologs	Targets	Function	Reference
Yeast			
Set2 (KMT3)	H3 K36 me1, me2, me3	Transcription elongation.	10
		Maintains histone stability over the coding regions.	69
		Prevents incorporation of acetylated histones.	69
		Stimulates deacetylase activity.	55, 56, 60, 61
C. elegans			
MES-4	H3 K36 me1, me2	X-chromosome silencing, germ cell viability.	100, 102, 103
		Germ cell viability.	100
MET-1	H3 K36 me3	Transcription elongation specific mark.	105
		Vulval development.	105
D. melanogaster			
dMES4	H3 K36 me1, me2	H4 K16 acetylation during transcription elongation.	86
HYPB/dSet2	H3 K36 me3	Transcription elongation	86
		Dosage compensation	84, 85
		Role in larval-pupal transition.	108
H. sapiens			
SETD2/HYPB/KMT3A	H3 K36 me3	Splicing.	93, 95, 96
NSD1/KMT3B	H3 K36 me1, me2, H4 K20 me3	Post-gastrulation embryonic development.	107
NSD2/WHSC1	H3 K36 me2	Cranio facial development.	71
		Heart development.	71
NSD3/WHSC1L1	H3 K36 me2		
ASH1L	H3 K36 me3, H3 K4 me		
SMYD2	H3 K36 me2	Regulation of p53 and Rb proteins.	53
SETMAR	H3 K36 me2	Non homologous end joining.	109
A. thaliana			
SDG8/EFS	H3 K36 me2, me3, H3 K9 me3	Stress response to fungal infections.	115
		Flowering response.	112, 113
		Anther and ovule development.	114
SDG26	H3 K36 me1	Flowering response.	112, 113

Table 2

Factors affecting Set2-mediated H3 K36 methylation

Factor	Effect on H3 K36 methylation	Reference
RNAPII and elongation factors		
RNAPII CTD Ser-5-P and S-2-P	Affects me2, me3	10, 23, 24, 25
CTDK1 (Ctk1, Ctk2, Ctk3)	Affects me1, me2, me3	26, 27, 28
Bur1	Affects me3	29, 33
Paf1C (Ctr9 and Paf1)	Affects me3	33
Factors affecting nucleosome stability		
Asf1	Affects me3	37
Spt6/Iws1	Affects me3	35, 36
FACT complex	Affects me3	40, 41
Histone H4 K44, H2B L116, L117	Affects me2, me3	21, 22
Histone H3 P38 and Fpr4 proline isomerase	Affects me3	43
Factors affecting RNA stability		
HnRNP-L (Human)	Affects me3	44
Histone H3 K36 Demethylases		
Jhd1	Affects me1, me2	47, 49, 50
Rph1	Affects me2, me3	47, 48, 50