HHS Public Access

Author manuscript

Trends Genet. Author manuscript; available in PMC 2024 November 01.

Published in final edited form as:

Trends Genet. 2023 November ; 39(11): 858–872. doi:10.1016/j.tig.2023.06.008.

Insights into Spt6: a histone chaperone that functions in transcription, DNA replication, and genome stability

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Abstract

Transcription elongation requires elaborate coordination between the transcriptional machinery and chromatin regulatory factors to successfully produce RNA while preserving the epigenetic landscape. Recent structural and genomic studies have highlighted that Spt6, a conserved histone chaperone and transcription elongation factor, sits at the crux of the transcription elongation process. Other recent studies have revealed that Spt6 also promotes DNA replication and genome integrity. Here we review recent studies of Spt6 that have provided new insights into the mechanisms by which Spt6 controls transcription and have revealed the breadth of Spt6 functions in eukaryotic cells.

Keywords

Spt6; histone chaperone; transcription elongation; chromatin; DNA replication; genome stability

Spt6 is a histone chaperone that is critically important throughout eukaryotes

Histone chaperones (see Glossary) are a large and diverse class of factors that establish and overcome nucleosomal barriers during DNA-templated processes. Many histone chaperones are essential for viability and have been implicated in human diseases [1–4]. A current challenge for the field is to elucidate the specific roles of the large number of histone chaperones associated with transcription, DNA replication, and DNA repair. This review focuses on advances in understanding one essential histone chaperone, **Spt6**.

Studies of Spt6 have revealed that it is critical throughout eukaryotes. First, Spt6 is essential for viability in most organisms in which it has been tested, from *Saccharomyces cerevisiae*

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Declaration of interests

The authors declare no conflict of interest.

to humans [5–11]. In humans, Spt6 has been linked to several types of cancer [12–16]. Additionally, depletion of Spt6 in mammalian cell culture models causes tissue specific defects [13,17–20]. Furthermore, from analysis of *spt6* mutants or by overexpression screens, Spt6 or its homologs are required for growth and development in other model organisms, including *Caenorhabditis elegans* [21], *Xenopus* [22], zebrafish [23,24], and *Arabidopsis* [25]. Combined, these results highlight the biological importance of Spt6 in eukaryotes and underscore the importance of Spt6 function for human health.

Since its discovery, Spt6 has been primarily studied for its roles in transcription. The crucial roles of Spt6 appear to be in promoting transcription elongation, regulating chromatin structure, and maintaining the fidelity of transcription initiation. Several recent studies have advanced our understanding of how Spt6 functions within the **RNA polymerase II (RNAPII)** elongation complex, addressed how Spt6 interacts with histones and **nucleosomes**, and discovered previously unidentified roles for Spt6, most prominently in DNA replication.

Spt6 functions as part of the RNAPII transcription elongation complex

Spt6 is a large multifunctional protein with seven domains (Figure 1A, 1B). The central core region, conserved in bacteria [26–29], is flanked by N- and C-terminal domains that are found only in eukaryotes. The N-terminal domain is highly acidic, largely intrinsically disordered, and as described below, necessary for Spt6 to interact directly with histones. The C-terminal region contains two **SH2 domains** that bind directly to RNAPII and facilitate the Spt6-RNAPII interaction. Additionally, Spt6 physically interacts with several other essential transcription elongation factors to promote transcription (Table 1). Recent structural studies have deepened our understanding of how Spt6 is positioned within the elongation complex.

Spt6 has multiple interactions with RNAPII

Spt6 interacts directly with RNAPII via the two Spt6 SH2 domains [30–32]. While early evidence pointed towards binding of the Spt6 SH2 domains to the carboxy-terminal domain (CTD) repeats of the RNAPII subunit Rpb1 [27,31,33–38], more recent studies have revealed that they bind to the Rpb1 CTD linker region [30,39,40]. This binding is enhanced by phosphorylation of the Rpb1 linker region by P-TEFb in mammalian cells and by Bur1 in *S. cerevisiae* [30,39,41]. This result also agrees with the demonstration that, in metazoans, Spt6 associates with actively transcribing RNAPII, but not with **promoter-proximally paused** RNAPII [42–46].

The importance of the Spt6 SH2 domains for binding RNAPII is strongly supported by *in vivo* experiments. While wild-type Spt6 co-localizes with elongating RNAPII across actively transcribed regions, Spt6 mutants lacking the SH2 domains, or with mutations in the SH2 domains, have greatly reduced chromatin association [30–32,40,44–56]. Furthermore, loss or mutation of the SH2 domains causes severe growth defects in *S. cerevisiae*, showing that Spt6 recruitment to chromatin is important for its function [30,34,35,38,40,52].

In addition to the Spt6 SH2-Rpb1 linker interaction, cryo-EM data have elucidated an additional Spt6-RNAPII interaction; the core region of Spt6 partially coats the outer surface

of RNAPII, binding to the Rpb4-Rpb7 stalk region [39,43,57] (Figure 1B, 1C). This Spt6-Rpb4-Rpb7 interaction likely contributes to transcription elongation as it repositions Rpb4-Rpb7 and secures the interaction of RNAPII with another elongation factor, the **DSIF complex (Spt4-Spt5 heterodimer)**, in order to open the RNA exit channel, as described in the next section.

Spt6 and DSIF (Spt4-Spt5) form the RNA exit channel

Since the discovery of Spt6, substantial genetic and biochemical evidence has connected it with DSIF. These studies, all in *S. cerevisiae*, demonstrated multiple genetic interactions between *spt4*, *spt5*, and *spt6* mutations [51,58–60], as well as Spt6-Spt5 physical interactions [59,60]. These results strongly suggested that these three proteins serve closely related roles during transcription elongation.

Cryo-EM structures of the mammalian RNAPII elongation complex have provided important insights into the physical interactions between Spt6 and DSIF. In this complex, Spt6 sits on top of DSIF, clamping DSIF to RNAPII [39,43,57] (Figure 1C). Several domains of Spt5, specifically KOW2-KOW3 and KOWx-KOW4, form the exit channel through which nascent RNA is extruded [61]. In the promoter-proximally paused elongation complex, when Spt6 is absent, these domains are in a closed conformation; then, in the activated elongation complex, with Spt6 present, these domains rotate, opening the RNA exit channel [39,43,57].The Spt6 S1 and Yqgf/RuvC domains also directly participate in the RNA exit channel and surround approximately eight nucleotides of the exiting RNA [39,43,62,63] (Figure 1C). In addition to conformational changes that open the RNA exit channel, DSIF domains at the DNA entry site are also altered upon Spt6 binding, although it is unclear whether this change promotes transcription [39].

Spt6 is required for the association of Paf1C with the RNAPII elongation complex

Similarly to DSIF, early genetic studies indicated that Spt6 and the **Paf1 complex (Paf1C)** contributed to a common transcription elongation function [54,64]. More recently, Spt6 has been shown to be required for Paf1C to associate with the RNAPII elongation complex [53,65,66], likely via an interaction between the Spt6 SH2 domains and the Cdc73 subunit of Paf1C [53,57] (Figure 1C). Notably, the RNAPII-Paf1C interaction is mutually exclusive with the interaction of RNAPII with NELF, a complex associated with promoter-proximally paused RNAPII, as they both occur on the same region of RNAPII [39,43,66]. Spt6 may help to promote the release of the elongation complex from the paused state by facilitating the exchange of Paf1C for NELF on RNAPII [66].

The physical and functional relationships between Spt6 and Iws1/Spn1

Spt6 physically interacts with another essential and conserved histone chaperone, **Iws1 (Spn1 in *S. cerevisiae*)** via a short alpha-helical region in the N-terminal domain of Spt6 [67–69] (Figure 1B). In *S. cerevisiae* the Spt6-Iws1 interaction has been shown to be strengthened by casein kinase II-dependent phosphorylation of Spt6 [70,71]. The Spt6-Iws1 interaction is clearly important, as Spt6 is required to recruit Iws1 to chromatin [51,56,67,68,72–74], and *spt6* mutations that impair Spt6-Iws1 binding cause strong transcription and chromatin defects [51,67,68].

In spite of the strong physical association between Spt6 and Iws1, they appear to function in related, but separate ways. For example, recent studies in *S. cerevisiae* showed that they cause distinct effects on transcription after their respective depletions, with Iws1 depletion causing less severe defects [49,74].

Spt6 functions in a histone chaperone network at the RNAPII-nucleosome interface

A recent series of cryo-EM structures of the RNAPII elongation complex transcribing through a nucleosome have shed light on how a histone chaperone network that contains Spt6 might coordinate nucleosome disassembly in front of, and nucleosome reassembly in the wake of, transcription [62,63,75,76]. In addition to Spt6, this network contains the histone chaperones Iws1 and **FACT**, as well as DSIF, which was recently suggested to have histone chaperone activity [77,78]. Functionally related roles for these four chaperones have been suggested by a wealth of genetic studies that have demonstrated common mutant phenotypes as well as allele-specific genetic interactions [51,59,79–85]. Below we review recent cryo-EM structures of elongating RNAPII as it enters and exits the nucleosome [63].

The RNAPII elongation complex as it transcribes into a nucleosome

As RNAPII approaches and enters a nucleosome, Spt6 and Iws1 sit at the RNAPII-nucleosome interface, with Iws1 directly bound to H3–H4 [63] (Figure 2A). While the flexible N-terminal tails of both Spt6 and Iws1 are not resolved in these structures, three results suggest that they are strong candidates to facilitate disruption of histone-DNA contacts in front of the approaching RNAPII: first, they are positioned adjacent to the exposed histone surfaces in one structure (Figure 2A); second, *in vitro* experiments have demonstrated that a short sequence within the Iws1 N-terminal region binds histones H3–H4 [86]; and third, the Spt6 N-terminal domain is necessary for Spt6 to bind to histones, as detailed below. As RNAPII progresses, FACT is observed to bind to the partially unwrapped nucleosome (Figure 2B), consistent with previous structural analysis of FACT [75,87].

The RNAPII elongation complex after transcription through a nucleosome

As RNAPII exits the nucleosome, structural analysis reveals a direct physical interaction between Spt6 and FACT (Figure 2C). In this structure, Spt6 binds FACT behind elongating RNAPII, with the Spt6 DLD and YqgF domains interacting with the middle domain of Spt16 (Figure 2C) [63]. This interaction bridges the FACT-bound sub-nucleosomal particle (lacking one H2A–H2B dimer) [87] with the Spt6-bound RNAPII [63]. DSIF also forms contacts with FACT and the exposed H3/H4 surface, supporting its recently identified role in chromatin maintenance [77,78]. Finally, after RNAPII has transcribed further past the nucleosome, FACT and Spt6 disengage from the mostly reassembled nucleosome, while DSIF maintains contact with the final H2A/H2B dimer to be rewrapped (Figure 2D). While these and other structures have provided unprecedented views of nucleosomal transcription, a major challenge for future studies will be to more fully elucidate the dynamics that occur among Spt6, the other chaperones, histones, and DNA during this process.

Spt6 binds histones and assembles nucleosomes

Spt6-histone interactions

Consistent with its role as a histone chaperone, Spt6 binds directly to histones and nucleosomes. Studies have shown that full-length yeast Spt6 (residues 1–1451) binds to both H2A–H2B dimers and H3–H4 tetramers *in vitro* [80,86,88,89], even when Spt6 is in a complex with Iws1 [86]. A yeast Spt6 derivative containing amino acids 237–1451 still binds to all four histones, but Spt6 297–1451 does not, showing a requirement for Spt6 amino acids 238–297 for histone binding [89]. Several important questions remain to be addressed regarding Spt6-histone interactions, including the possible role of the rest of the N-terminal end, whether different regions are responsible for binding different histones, and whether the N-terminal end is sufficient for Spt6 to bind to histones.

Spt6-nucleosome interactions

In vitro studies have shown that Spt6 is able to assemble and bind to nucleosomes [67,88,89]. However, the understanding of the Spt6-nucleosome interaction is uncertain as different studies have looked at different versions of Spt6 with respect to the amount of the N-terminal domain that was included. The current state of understanding is: (1) the binding of Spt6 to nucleosomes requires Nhp6 [67], the same protein required by FACT to bind to nucleosomes [90,91]; (2) Spt6 competes with Iws1 for binding to nucleosomes, but not for binding to histones [67,86]; (3) the region of Spt6 required for nucleosome binding is distinct from that required for histone binding [86,89]; (4) the requirement for the Spt6 N-terminal domain in binding to nucleosomes remains uncertain, as it was required in one study but not another [67,89]; and (5) the binding of Spt6 to nucleosomes (and to RNAPII) may be regulated by intramolecular interactions between the Spt6 N- and C-terminal domains [89]. A more complete understanding of Spt6-nucleosome interactions will require a systematic analysis, testing the requirements for different regions of Spt6, the roles of Iws1 and Nhp6, and whether Spt6 primarily binds to a partially unwrapped nucleosome as previously shown for FACT [87].

Spt6 regulates chromatin structure

Spt6 controls nucleosome occupancy and positioning

Several genetic studies [6,7,88], along with the evidence for Spt6-histone interactions and Spt6 nucleosome assembly activity described above, suggested that Spt6 controls chromatin structure *in vivo*. This conclusion was further supported by studies that showed that *spt6* mutants in both *S. cerevisiae* and *Schizosaccharomyces pombe* impaired nucleosome positioning [50,92–94].

Recent high-resolution genomic studies in *S. cerevisiae* have provided additional insights into the requirement for Spt6 in the control of chromatin structure. Upon Spt6 depletion, the level of nucleosomes is greatly decreased across the genome and the remaining nucleosomes are less well positioned (“fuzzier”) [49,95], consistent with a role in reassembling nucleosomes in the wake of transcription. Furthermore, in one *spt6* mutant, the intra-nucleosomal distance is increased, indicating that Spt6 controls nucleosome spacing [51].

How are the changes in chromatin structure in *spt6* mutants related to transcription? One early study demonstrated that, when Spt6 was depleted, chromatin disruption over the *GAL1* gene required transcription, consistent with a model in which Spt6 restores chromatin in the wake of transcription [96]. However, the level of transcription may not be important for this effect, as a recent genome-wide study showed that the effect of Spt6 depletion on chromatin structure is independent of transcription levels [49].

Spt6 regulates histone post-translational modifications

Spt6 is also required for several histone **post-translational modifications (PTMs)**, some of which are species specific (summarized in Table 2). The most extensively studied requirement for Spt6 is in H3K36 methylation. Several early studies in *S. cerevisiae* revealed that in some *spt6* mutants, including depletion of Spt6, the level of H3K36 di- and trimethylation was greatly reduced [97–99]. Furthermore, prolonged depletion of Spt6 in mammalian cells causes a redistribution of H3K36 trimethylation such that it is reduced across gene bodies and increased at noncoding RNA loci, reflecting the transcriptional changes [100]. There appear to be at least two mechanisms by which Spt6 controls H3K36 methylation. First, under some conditions, Spt6 is required for normal levels of the H3K36 methyltransferase Set2 (SetD2 in humans) [98,101,102]. More recent work has shown that Spt6 also regulates Set2 activity through modulation of a Set2 auto-inhibitory domain [102,103]; however, the mechanism by which this occurs is unknown. Spt6-dependence of H3K36 methylation is conserved in mammalian cells as Spt6 and Iws1 are required for recruitment of SetD2 [73,104].

Dissection of the roles of Spt6 during transcription

Although Spt6 was identified by the isolation of mutations that suppress transcription initiation defects [5,58,105], subsequent work showed that the primary role for Spt6 is in transcription elongation [106]. In addition, Spt6 also controls other aspects of transcription, including regulation of intragenic transcription and transcription termination.

Spt6 promotes transcription elongation

Early results showed that Spt6 controls transcription elongation both *in vitro* [107] and *in vivo* [108]. Recent mutli-omics studies in human cells have begun to dissect how Spt6 controls transcription elongation, by rapidly depleting Spt6 with a degron-tag and examining the consequences [20,66,109,110]. Upon Spt6 depletion, RNAPII accumulates at the 5’ end of genes, past the promoter proximal pause site but near the +1 nucleosome [109,110], and it is lost from the 3’ ends of genes [66,109,110]. Correspondingly, transcription is reduced across gene bodies [109,110], a result previously seen in *S. cerevisiae* [49,83] (Figure 3). This defect is more extreme for long genes with respect to both RNAPII occupancy and RNA production, a result that is most consistent with defective processivity in the absence of Spt6 [66,109,110]. Mathematical modeling of these genomic data suggested that in the absence of Spt6 the rate or velocity of RNAPII is decreased by up to 25% [109]. Prolonged Spt6 loss following siRNA treatment had similar defects in transcription elongation, as RNA was lost from gene bodies although RNAPII occupancy was not examined [100].

Multiple models, not mutually exclusive, can explain how Spt6 promotes RNAPII elongation. First, current evidence supports a model in which Spt6 assists RNAPII transcription through nucleosomal barriers. This is consistent with the 5' accumulation of RNAPII at +1 nucleosomes after Spt6 depletion [109,110], as well as the suppression of yeast *spt6* mutant phenotypes by histone mutations that destabilize nucleosome interfaces [80]. Furthermore, while Spt6 is not required *in vitro* for transcription of a nucleosomal template, it does accelerate the rate of transcription [110]. Second, Spt6 is required to recruit and/or stabilize additional factors within the transcription elongation complex that contribute to elongation, including Paf1C [53,65,66,111,112]; therefore, loss of some of these factors may contribute to the elongation defects observed after Spt6 depletion. Finally, as described earlier, the binding of Spt6 to RNAPII may alter RNAPII structure to promote processivity [39,42], although this remains to be determined. Given the multiple interactions and functions of Spt6, it seems most plausible that a combination of these models contributes to the critical function of Spt6 during transcription elongation.

Spt6 represses intragenic transcription initiation

One of the most surprising results from analysis of yeast *spt6* mutants was the demonstration that they allow **intragenic transcription** initiation from thousands of sites [49,52,82,92,96,113]. These effects are similarly observed following prolonged Spt6 loss in mammalian cells, in which there is increased expression of not only intragenic transcripts, but also enhancer RNAs (eRNAs), long non-coding RNAs (lncRNAs), and antisense promoter upstream transcripts (PROMPTs) [100,109].

Spt6 likely controls intragenic initiation by multiple mechanisms. One established mechanism is through Spt6-dependent H3K36 di- and trimethylation, as studies have shown that loss of H3K36 methylation allowed intragenic transcription in *S. cerevisiae* [114,115]. Similarly, redistribution of H3K36 methylation caused by prolonged loss of Spt6 promotes non-coding transcripts in mammalian cells [100]. This cannot be the only mechanism, however, as the degree of intragenic transcription is greater after Spt6 depletion than after loss of Set2 [49]. Furthermore, intragenic transcription also occurs in an *spt6* mutant that has no detectable effect on H3K36 methylation levels [51]. Another likely mechanism is via Spt6 control of chromatin structure, as *spt6* mutants display decreased nucleosome occupancy and positioning [49,52,71,82,92,93,96,109,113], thereby exposing intragenic initiation sites within gene bodies [49].

There has been considerable speculation regarding possible functions for intragenic transcription. One possibility is that intragenic transcripts encode proteins with altered N-termini, thus providing alternate genetic information, similar to alternative splicing. Indeed, there is substantial evidence that many intragenic transcripts are translated (for example [82,116]), some with clearly different functions from their full-length counterparts (for example [117]). However, as several thousand intragenic promoters become active following Spt6 depletion, it seems unlikely that most intragenic transcripts encode functional proteins and more likely that most of them are non-functional.

Spt6 and transcription termination defects

The recent studies of Spt6 depletion in human cells have suggested that Spt6 regulates RNAPII transcription termination, although the specific effects appear to be dependent upon the length of Spt6 depletion and the class of transcript. In one study, prolonged depletion of Spt6 in human cells led to the failure of RNAPII to transcribe the full lengths of some protein coding genes, resulting in pre-mature termination [100]. Interestingly, the same study showed that prolonged Spt6 depletion caused the opposite effect at lncRNA genes, with elevated transcription and 3' read-through, possibly due to reduced recruitment of the Integrator complex [100]. In contrast to prolonged Spt6 depletion, another study showed that rapid Spt6 depletion led to read-through transcription 3' of the poly-adenylation site for thousands of protein coding genes [109]. Additional experiments suggested that this termination defect was caused by reduced recruitment of 3' cleavage and termination factors [66,100,109].

Other roles for Spt6 in transcription

Other studies have shown that Spt6 has additional functions in transcription, with more likely to emerge. First, Spt6 regulates the level of transcription initiation at specific genes, likely by controlling promoter chromatin structure [40,50,118], and at many more genes, likely due to the dilution of initiation factors over thousands of intragenic promoters [49]. In addition, Spt6 affects co-transcriptional mRNA processing [33,40,52,65,66]. Finally, Spt6 may regulate transcription by other RNA polymerases, as evidence exists for Spt6 control of RNAPI transcription and ribosome biogenesis [119,120], as well as control of RNAPIII transcription [121].

Spt6 is required for genome stability and DNA replication

Many studies have shown that Spt6 is important in controlling DNA damage and **genome stability**. The initial evidence for the role of Spt6 in promoting genome stability came from the demonstration that *S. cerevisiae spt6* mutants had increased levels of recombination and chromosomal loss [122–124]. More recent experiments in both *S. cerevisiae* and mammalian cells have identified additional genome instability phenotypes caused by the loss of Spt6, and have suggested possible mechanisms by which Spt6 might regulate genome stability.

Spt6 may be important for genome stability through its many roles in regulating transcription and chromatin structure, similar to what has been observed for other histone chaperones [125]. As a key regulator of transcription, loss of Spt6 may decrease the expression of DNA repair genes or may increase toxic by-products of transcription. For example, Spt6 is required for the expression of the error-free DNA damage repair factor BRCA1 in glioblastoma cancer-stem like cells [14]. Loss of Spt6 also impairs stem cell self-renewal, identifying Spt6 as a potential cancer therapeutic target [14]. Additionally, Spt6 may control genome stability by the regulation of transcription termination, as described in the previous section [100,109]. The presence of extended transcripts after Spt6 depletion, for either protein-coding or lncRNA genes, may lead to an increase in transcription-replication conflicts. In support of this hypothesis, Spt6 loss from mammalian cells led to an increased level of γ-H2AX, a marker of DNA damage and replication stress [100,109]. In both

studies, **R-loops** are suggested to be important in promoting DNA damage [100]. However, one of those studies [100], as well a study of yeast *spt6* mutants, showed that over protein-coding genes, the level of R-loops is decreased [100,126], reflecting the general loss of transcription over these regions. Therefore, it remains to be determined, particularly in yeast *spt6* mutants, whether R-loops directly contribute to the observed increase in DNA damage. Alternatively, Spt6 may regulate genome integrity by regulating chromatin structure. Of particular interest is the Spt6-dependence of H3K36 methylation, which plays a large role in the DNA damage response [127]. While broad effects of H3K36 methylation loss following Spt6 depletion have not been investigated, Spt6-dependent deposition of H3K36 methylation is required for immunoglobulin class-switch recombination in human cells, which requires an induced DNA double strand break [128,129]. Transcription and chromatin defects alone cannot fully explain genome instability in yeast *spt6* mutants however, as at least one mutant with strong instability phenotypes has few transcriptional changes, a global decrease in R-loops, and normal levels of H3K36 methylation [51,126].

Recent experiments in *S. cerevisiae* and mammalian cells have shown that Spt6 is required for DNA replication, suggesting that genome instability upon loss of Spt6 might be explained, at least in part, by defects in DNA replication [109,126]. Depletion of Spt6 results in a dramatic decrease in DNA synthesis as measured by the incorporation of the nucleotide analog BrdU in both systems [109,126]. More extensive studies in *S. cerevisiae* revealed that *spt6* mutants are extremely sensitive to DNA replication inhibitors [51,52,126], have negative genetic interactions with mutations that impair the DNA replication machinery and S-phase checkpoints [126], and have an increased level of double-strand breaks [126]. Experiments suggest that the requirement for Spt6 during DNA replication occurs during origin licensing, as *spt6* mutants have impaired loading of the MCM replicative helicase [126]. Given this newly identified function of Spt6 and its importance for genome integrity, it will be important to determine if Spt6 directly interacts with the replication machinery to promote origin licensing or if Spt6 acts more indirectly, by controlling chromatin structure to promote MCM loading. Furthermore, it will be interesting to see if Spt6 also affects origin licensing in metazoans where replication origins are less clearly defined.

Concluding remarks

Although eukaryotic transcription has been intensively studied for decades, important new discoveries continue to emerge. One of these is the critical requirement for multiple histone chaperones during transcription elongation. Central among these is the essential histone chaperone Spt6, along with Iws1, FACT, and Spt5. While studies reviewed here have begun to decipher possible specific interactions between and functions of each of these factors during transcription, it is still not clear why so many are necessary (see Outstanding questions). This suggests that additional essential aspects of transcription on a chromatin template are yet to be discovered.

While Spt6 was discovered as a transcription factor, it is involved in multiple chromatin-related processes (Figure 4), a characteristic also true of many other histone chaperones. The multi-purposing of histone chaperones may have evolved as an economy of resources, but it may also reflect a cellular need to coordinate fundamental DNA-templated processes,

transcription, replication, and repair, in order to avoid catastrophic events leading to genome instability. As genome instability is a hallmark of cancer, and as histone chaperones become potential therapeutic targets in cancer treatment [130], fully understanding the role of Spt6 and other histone chaperones in maintaining genome integrity will be of great importance.

Acknowledgments

We thank Karen Arndt and Elmar Wolf for helpful comments on the manuscript. This work was supported by NIH grant R01GM135251 to F.W.

Glossary

DSIF complex
DSIF (DRB-sensitivity inducing factor) is a heterodimer of Spt4 and Spt5. It as a critical regulator of transcription elongation and promotor-proximal pausing. Recent evidence has also suggested that DSIF has histone chaperone activity. DRB (5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole) is a chemical inhibitor of transcription elongation.

FACT
FACT (Facilitates chromatin transactions) is histone chaperone complex that is a heterodimer of Spt16 and SSRP1 in humans and Spt16 and Pob3 in yeast. FACT components were initially identified in yeast and subsequently in humans as important for transcription on chromatin templates, as well as for DNA replication.

Genome stability
The ability of a cell to prevent an increase in chromosomal abnormalities or DNA mutations, often driven by an increase in DNA damage or errors in DNA replication

Histone chaperone
A protein/complex that directly interacts with histones, nucleosomes, or sub-nucleosomal particles in an ATP-independent manner. Histone chaperons can regulate the entire life-cycle of histones, including nuclear import, assembly onto chromatin, and post-translational modification.

Histone post-translational modifications (PTMs)
A large range of modifications, including but not limited to acetylation, methylation, and ubiquitination, of the histone core, N-terminal flexible tails, or C-termini that can alter the chemical interactions of the nucleosome and result in altered histone-DNA or histone-histone interactions.

Intragenic transcription
The expression of transcripts from non-canonical promoters resulting in antisense and intragenic transcripts initiating within a gene body.

Iws1 (Spn1 in *S. cerevisiae*)
Iws1 is an essential and conserved histone chaperone with roles in transcription elongation and mRNA biogenesis.

Nucleosome
A protein-DNA complex consisting of ~147 base-pairs of DNA wrapped around an octameric histone core (containing one H3–H4 tetramer and two H2A–H2B dimers).

Paf1 complex (Paf1C)
Paf1C (Polymerase-associated factor complex) contains Paf1, Ctr9, Leo1, and Cdc73. It plays key roles in transcription elongation, histone methylation, RNA processing, and RNAPII release from promoter-proximal pausing. In *S. cerevisiae* Paf1C also contains the Rtf1 subunit; however, in other organisms, Rtf1 does not associate strongly with Paf1C and it has been shown to have independent functions.

Promoter-proximal pausing
A highly regulated step during metazoan transcription in which the engaged RNAPII stably pauses just downstream of the promoter. While paused, RNAPII associates with negative elongation factor (NELF).

R-loops
Long (>100 bp) nucleic acid secondary structure consisting of an RNA:DNA hybrid and the resulting ssDNA strand that form over transcribed gene regions. They can have regulatory and hazardous roles within the cell.

RNA polymerase II (RNAPII)
RNA polymerase II is one of three RNA polymerases found in eukaryotes. RNAPII is primarily responsible for producing mRNAs.

SH2 domain
Src homology 2 (SH2) domain is a conserved protein domain that binds to phosphorylated tyrosines. The only SH2 domains in *S. cerevisiae* are those in the C-terminus of Spt6. The Spt6 SH2 domains are unusual in that they can also bind phosphorylated serines.

Spt6
Spt6 (Suppressor of Ty 6) is a conserved histone chaperone and transcription elongation factor. It is one of several factors identified that, when mutant, suppress the transcriptional defect caused by insertion of the Ty transposon (LTR or δ element) in the 5’ region of a reporter gene.

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Outstanding questions

- What is the nature of the interplay between Spt6, Iws1, DSIF, and FACT during transcription through a nucleosome? In what ways does each histone chaperone contribute to this process?
- How does Spt6 bind to histones and nucleosomes? Do distinct regions of Spt6 bind H2A–H2B and H3–H4?
- Does Spt6 control genome stability via its roles in transcription, replication, or both?
- What is the mechanism by which Spt6 is required for DNA replication? Does it direction interact with components of the replication machinery or does it control replication via controlling chromatin structure over replication origins?

Highlights

Spt6 is a structural hub of the activated RNA polymerase II (RNAPII) elongation complex and forms critical contacts with RNAPII as well as other elongation factors, including DSIF, Paf1C, Iws1, and FACT.

- Spt6 directly interacts with all four core histones and nucleosomes, and can assemble nucleosomes *in vitro*.
- Spt6 promotes processivity and elongation rate past nucleosomal barriers.
- Spt6 regulates nucleosome positioning and several histone post-translational modifications, thereby repressing intragenic transcription.
- Spt6 is necessary for genome stability, likely due to its roles in both transcription and DNA replication.

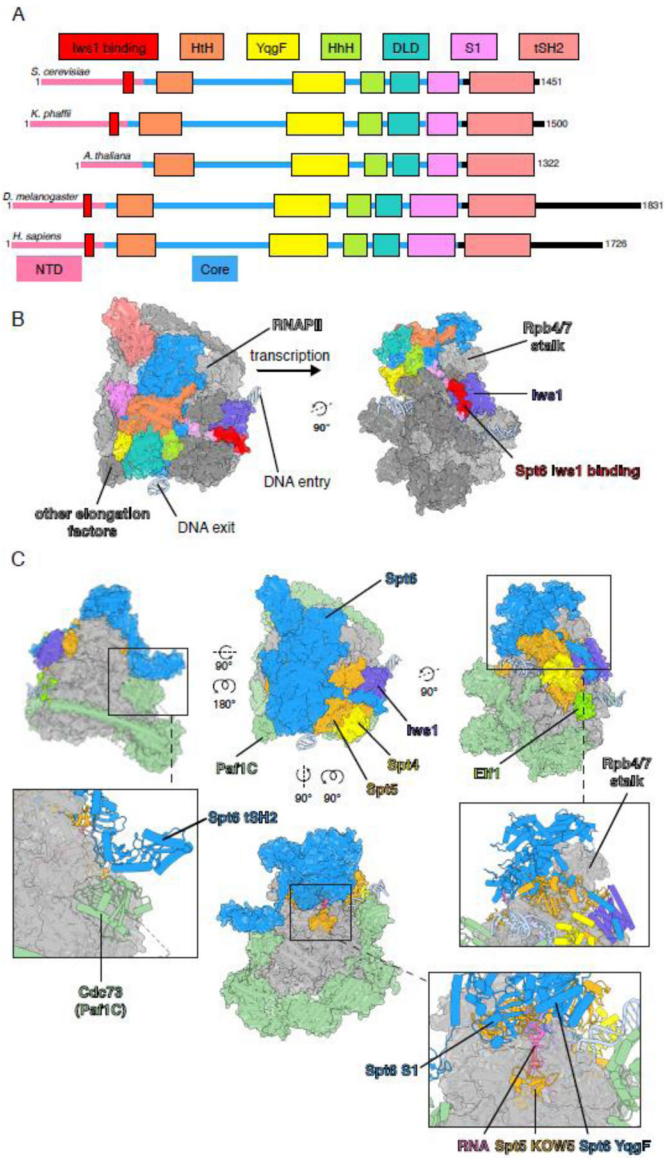

Figure 1. Spt6 is a core elongation factor with multiple domains and multiple interactions with the RNAPII transcription elongation complex.

(A) A diagram showing the alignment of the annotated domains of Spt6 [27] in multiple eukaryotic species: *Saccharomyces cerevisiae*, *Komagataella phaffii* (used in the structures below [63]), *Arabidopsis thaliana*, *Drosophila melanogaster*, and *Homo sapiens*. The blue connecting line indicates the Spt6 core region, which we have extended to include the S1 domain. The pink connecting line indicates the N-terminal domain (NTD). Amino- and carboxy-terminal amino acid residue numbers are indicated to the left and right of each homolog, respectively. (B) Cryo-EM structure of Spt6 in the RNAPII elongation complex (PDB: 7XN7) [63] with the domains of Spt6 color-coded to match the diagram in (A). The arrow indicates the direction of transcription. The Rpb4/7 stalk is labeled to show engagement by the Spt6 core region. The small portion of the Spt6 N-terminal domain in the structure (red) interacts with Iws1 (purple). The axis of rotation between the left and right perspectives is noted. (C) Cryo-EM structure (PDB: 7XN7) [63] of the RNAPII

transcription elongation complex with elongation factors colored. In these images, all of Spt6 is shown in blue. The images to the left show the Spt6 tSH2 domain engaging with the Paf1C subunit Cdc73 at the Rpb1 C-terminal domain linker (unresolved in this structure). The images below, middle and right, show the Spt6 YqgF and S1 domains and the Spt5 KOW5 domain cooperating to form the RNA exit channel. The image to the right shows the Spt6 core clamping DSIF (Spt4 and Spt5) to RNAPII. The axis of rotation is denoted between the central structure and each different perspective. Additional rotations have been indicated where necessary to maintain the relative position of Spt6 on top of the transcription elongation complex.

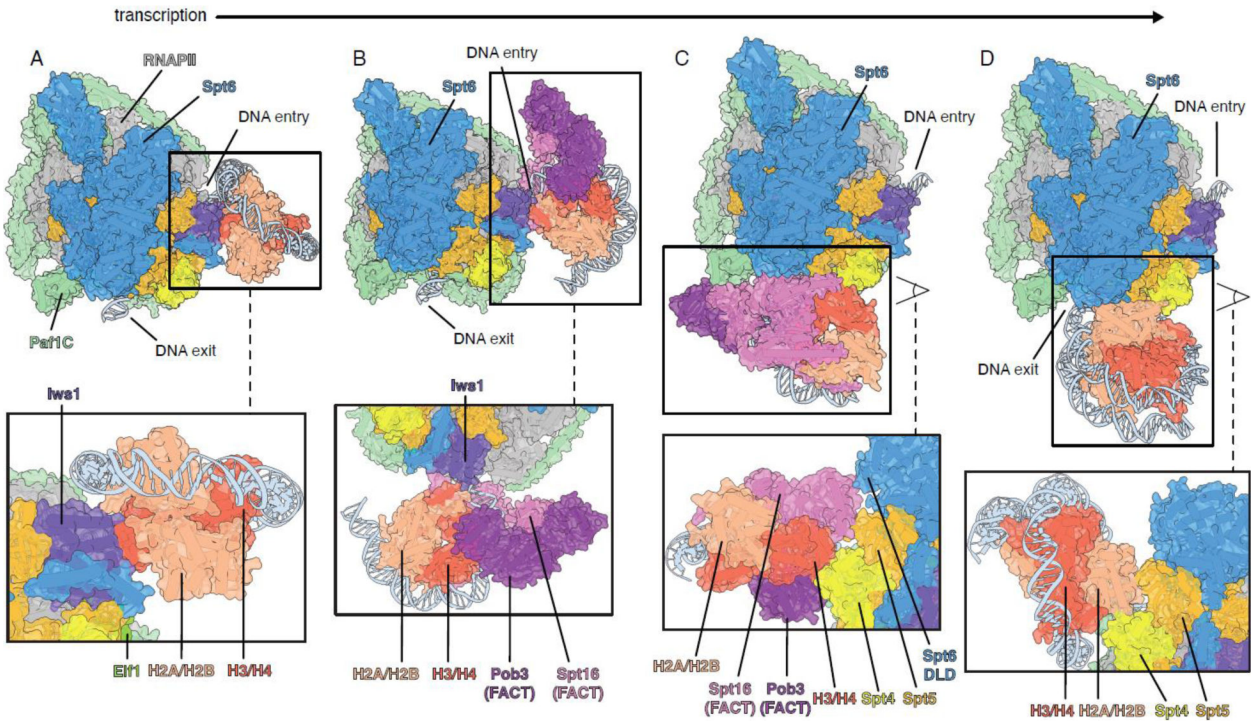

Figure 2. Spt6 functions in a histone chaperone network as RNAPII transcribes through a nucleosome.

A series of structures [63] showing the disassembly and reassembly of a nucleosome by the RNAPII transcription elongation complex. The elongation complex is shown in the same orientation in the top images, with transcription progressing from left to right (arrow). (A) As the elongation complex begins to unwrap the nucleosome (stalled 42 bp past nucleosome entry, PDB: 7XSE), Spt6 (blue) and Iws1 (purple) engage with the entry H2A/H2B dimer (salmon) and H3/H4 dimer (orange), respectively. The inset image shows the proximity of the Spt6 N-terminal domain and Iws1 to the exposed histone surface. (B) As the nucleosome is further unwrapped by the elongation complex (stalled 49 bp past nucleosome entry, PDB: 7XSX), contacts between Spt6 (blue), Iws1 (purple), and histones are no longer resolved. Instead, the FACT histone chaperone complex (Spt16, light pink; Pob3, violet) is now bound to the downstream nucleosome. The inset image shows that the nucleosome has rotated relative to its position in (A). (C) The elongation complex has transcribed past the dyad, and the nucleosome is now resolved, being reassembled behind RNAPII (stalled 58 bp past nucleosome entry, PDB: 7XTI). FACT (Spt16, light pink; Pob3, violet) remains bound to the sub-nucleosomal particle lacking one H2A/H2B dimer and has contacts with Spt6 (blue), DSIF (yellow and orange), and Paf1C (green). Spt4 (yellow) engages the exposed H3/H4 dimer (orange). The inset shows another view of these contacts. (D) The elongation complex has transcribed through and reassembled the nucleosome (stalled 115 bp past nucleosome entry, PDB: 7XSZ). Spt6 (blue) and FACT are no longer associated with the nucleosome; the final contacts are between DSIF (yellow and orange) and the exit H2A/H2B dimer (salmon). The inset shows another view of these contacts.

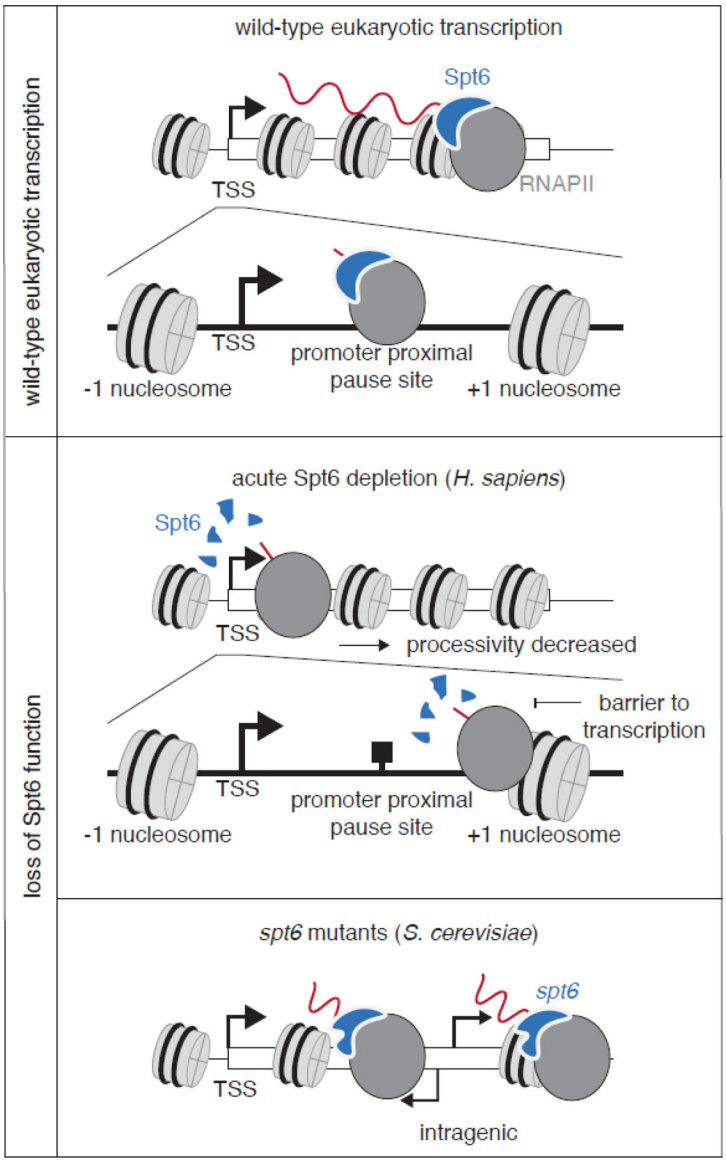

Figure 3. Roles of Spt6 during transcription elongation.

(A) In wild-type yeast or mammalian cells, Spt6 (blue) associates with the elongating RNAPII elongation complex (gray) to promote the production of RNA (red). In metazoans, promoter-proximal pausing (which does not occur *S. cerevisiae*) occurs between the +1 and −1 nucleosomes downstream of the transcription start site (TSS). (B) Acute loss of Spt6 from mammalian systems causes the accumulation of RNAPII at the 5' ends of genes and results in decreased RNA production. This defect is in part due to decreases in RNAPII processivity and elongation rate throughout the gene body. The accumulation of RNAPII in the 5' gene region appears to be at the +1 nucleosome rather than at the promoter-proximal pause site. (C) In *S. cerevisiae, spt6* mutants such as *spt6-YW* retain Spt6 levels, while others such as *spt6-1004* cause depletion of Spt6 protein. After Spt6 depletion, there is a global decrease in nucleosome occupancy, and in all mutants tested,

there is altered nucleosome positioning, a reduction in genic transcription, and a striking increase in intragenic initiation.

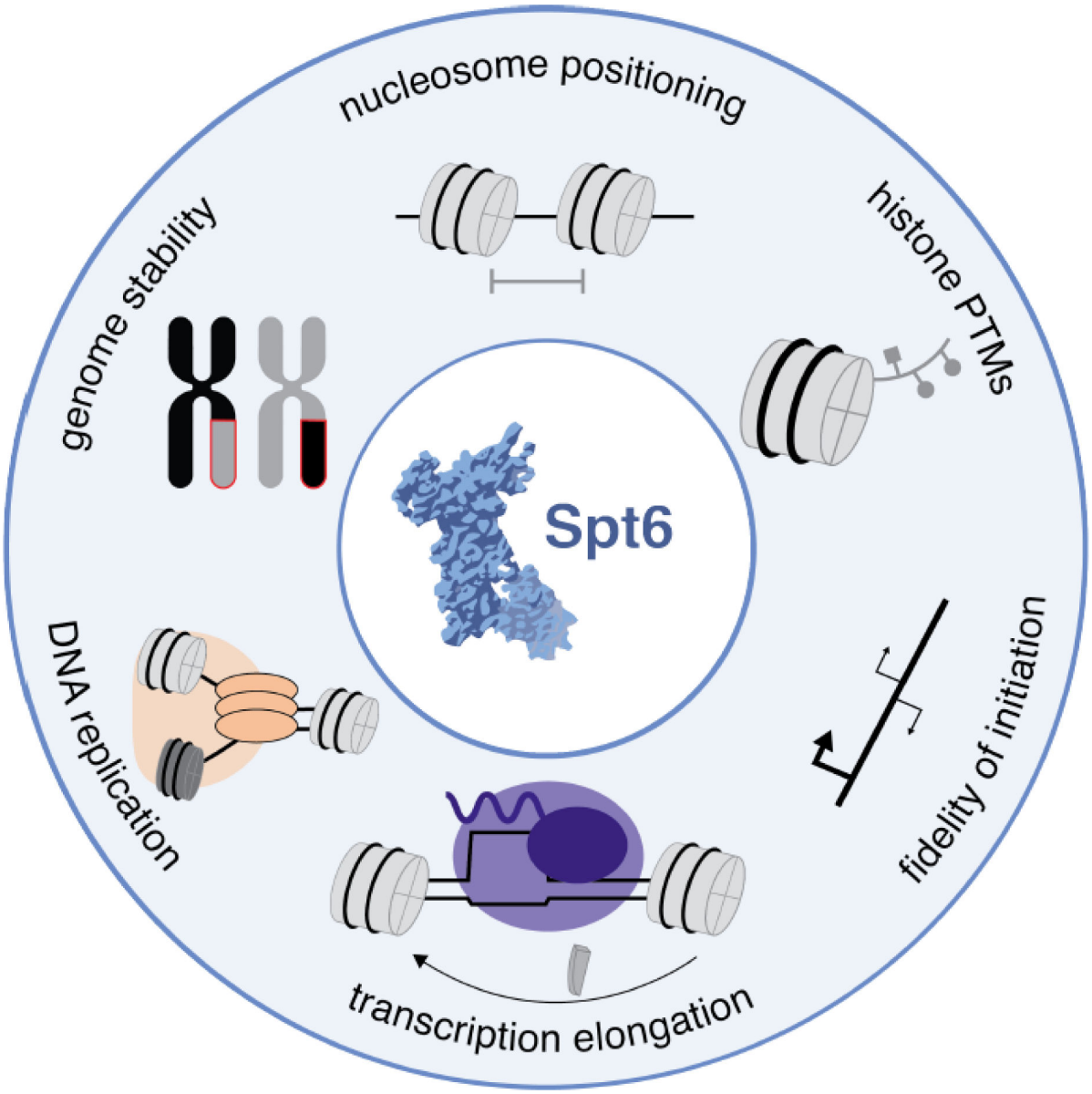

Figure 4. Spt6 is a multifunctional histone chaperone.

The diagram conveys the many functions of Spt6. Spt6 acts as a histone chaperone to promote nucleosome occupancy and positioning, and proper histone post-translational modification (PTMs). Spt6 is also a transcription factor that promotes transcription elongation along a chromatin template and regulates the fidelity of transcription initiation. More recent work has demonstrated that Spt6 is also required for DNA replication. Through a combination of these functions, Spt6 is a critical regulator of genome stability and acts as a caretaker of eukaryotic chromatin.

Table 1.

Summary of known Spt6 genetic, biochemical, and structural interactions.

Protein	Protein category	Evidence of interaction	Region of Spt6 involved	References
Rpb1	RNAPII	Genetic, biochemical, structural	SH2 domains	[27,33–35,38,131] [30,39,40]
Rpb4-Rpb7 stalk	RNAP II	Structural	Core region	[39,43,62,63]
Iws1 (Spn1)	Histone chaperone, transcription elongation factor	Genetic, biochemical, structural	N-terminal region	[56,67–69,72,73]
DSIF (Spt4-Spt5)	Transcription elongation factor	Genetic, biochemical, structural	Core region	[59,60]
Paf1C	Transcription elongation factor	Genetic, structural, biochemical	C-terminal region	[53,54,64,65]
FACT complex	Histone chaperone	Genetic, biochemical, structural	DLD, YqgF	[51,63,80,106,132,133]
Spt2	Histone chaperone	Genetic	unknown	[112,134]
Elf1	Transcription elongation factor	Genetic	N-terminal region	[111]
TFIIS	Transcription elongation factor	Genetic	unknown	[60]

Table 2.

Summary of chromatin alterations in *spt6* mutants or Spt6 depletion.

Chromatin mark	Organism/mutant	*spt6* mutant phenotype	Reference
Histone post-translational modifications (PTMs)			
H3K4 methylation (promoter associated mark)	*S. pombe*	No detectable H3K4me3	[92,135]
	Drosophila melanogaster, mouse, human	Decreased H3K4me3	[128,136]
	S. cerevisiae	Shift in H3K4me from promoter region to within gene bodies	[95]
H3K36 methylation (histone mark associated with active transcription)	*S. cerevisiae* and *S. pombe*; loss of HhH domain mutants *spt6-1004* and *spt6-1*	Complete loss of H3K36me2 and H3K36me3	[92,97–99,102,119]
	S. cerevisiae; spt6-14 and *spt6-50*	Loss of H3k36me3, H3K36me2 retained	[101]
	S. cerevisiae spt6-YW and human Spt6 depletion	H3K36me3 levels unaffected or only modestly reduced	[18,51,99,100,128]
	Human Spt6 prolonged depletion	Shift of H3K36me3; decrease within protein coding genes and increased within non-coding loci	[100]
H3K27 acetylation and methylation (metazoan specific mark associated with promoter and enhancer regions)	Human	Promotes H3K27 demethylation through interactions with the demethyltransferases (JMJD3 and KDM6A) or PRC2	[18,19,137]
H3K9 methylation (heterochromatin associated mark)	*S. pombe*	H3K9me3 decreased at heterochromatin regions	[10,135]
Histone Variants			
CENP-A (centromere associated histone variant)	*S. pombe*	Accumulation of CENP-A at non-centromeric regions	[138]
	D. melanogaster or human Spt6 depletion	Loss of CENP-A from centromeric regions	[139]
H2A.Z (promoter associated histone variant)	*S. cerevisiae*	H2A.Z accumulation in ectopic locations	[140]