Control of histone methylation and genome stability by PTIP

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PTIP regulates gene transcription by controlling the methylation of histone H3, and also has important roles in cellular responses to DNA damage or to perturbed DNA replication. The available data suggest that the functions of PTIP in transcription and preserving genome stability might be independent and mediated by functionally distinct cellular pools of PTIP. Although considerable progress has been made in understanding how PTIP influences transcription, a coherent picture of how it protects cells from DNA damage at the molecular level has yet to emerge. Here, we describe recent progress made in understanding the cellular roles of PTIP and the relevance of PTIP-interacting proteins, as well as the questions that have yet to be answered.

Keywords: 53BP1; ATM; DNA damage; histone methylation; MLL; PTIP

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See Glossary for abbreviations used in this article.

Introduction

Pax genes encode developmentally regulated transcription factors that have crucial roles in embryogenesis (Dahl et al., 1997). Mutations in Pax2 cause eye and kidney abnormalities (Sanyanusin et al., 1995; Devriendt et al., 1998), and Pax2-null mice are viable but lack kidneys and genital tracts (Torres et al., 1995). PTIP was initially discovered in a yeast two-hybrid screen for factors that interact with Pax2 (Lechner et al., 2000), and it was subsequently shown that the transcription of several Pax2-regulated genes depends on PTIP (Patel et al., 2007). In Xenopus laevis embryos, Ptip—also known as Swift—interacts with Smad2, which is part of the Smad2–Smad4 complex that binds to gene promoters and activates transcription in response to activin or TGF-β (Massague & Gomis, 2006). When over-expressed, Ptip stimulates Smad2-dependent transcription, whereas a mutant form of Ptip, which is unable to interact with Smad2, inhibits Smad2-dependent transcription and mesoderm development (Shimizu et al., 2001). These results indicate that PTIP stimulates PAX2-dependent and Smad2-dependent gene expression, and the subsequent observation that PTIP can regulate histone methylation has begun to explain the underlying mechanisms.

PTIP and histone methylation

Histone methylation is an important epigenetic mechanism that can regulate transcription; the methylation of H3K4, for example, is associated with transcriptional activation (Kouzarides, 2007). The evolutionarily conserved SET domain is a characteristic of the seven families of proteins with lysine methyltransferase activity, which are exemplified by the SET1 multiprotein complex. In humans, several SET1-like complexes have been identified, each containing one or two SET domain proteins—such as SET1, MLL1, MLL2/ALR, MLL3/HALR and MLL4— that are responsible for the enzymatic activity of the complex (Ruthenburg et al., 2007). All MLL complexes share the core subunits ASH2, RBQ3 and WDR5, which contribute to the structural scaffolding of SET1-like complexes and influence their substrate specificity (Ruthenburg et al., 2007). Importantly, PTIP has also been found to be an integral component of several such HMTase complexes (Fig 1) that differ in the catalytic subunits they contain—MLL2 (Issaeva et al., 2007), MLL3 plus MLL4 (Cho et al., 2007) or MLL2 plus MLL3 (Patel et al., 2007). Consistent with these observations, anti-PTIP immunoprecipitates contain H3K4—and H3K9—methyltransferase activity (Cho et al., 2007; Issaeva et al., 2007; Patel et al., 2007). The differences in the catalytic components of the PTIP complexes identified in these studies might indicate that PTIP associates with a core subunit that is common to all MLL complexes, or might reflect the differential expression or differences in the abundance of the MLL catalytic subunits in various cell types.

Although PTIP associates with MLL-containing HMTase complexes, it is not required for their enzymatic activity (Cho et al., 2007). Instead, PTIP is believed to recruit HMTase complexes to gene promoters by interacting with promoter-bound transcription factors (Fig 1). Chromatin-immunoprecipitation analyses have shown that PTIP associates with gene promoters that are known to be regulated by MLL2 (Issaeva et al., 2007). PAX2 recruits PTIP to PAX2-response elements in gene promoters and PTIP, in turn, recruits an MLL2-containing complex to these promoters (Patel et al., 2007). Furthermore, the depletion of PTIP from human cells prevents PAX2-dependent gene transcription, and the conditional ablation of Ptip from the developing spinal cord of Ptipflox/floxembryos results in a global decrease in the levels of methylated H3K4 (Patel et al., 2007). In addition, the conditional deletion of
Ptip in the renal inner medulla of mice results in renal function defects similar to those associated with defects in Pax2 function (Kim et al., 2007). Therefore, the available data strongly support a model in which PTIP interacts with transcription factors that bind directly to promoters—such as Pax2—and facilitates the recruitment of HMTase complexes, thereby activating gene transcription. PTIP was found to be an integral component of several SET-domain-containing HMTase complexes that exist in the various cell types used in these studies. H3K4, lysine 4 of histone H3; Me, methyl; MLL, mixed-lineage leukaemia; ORF, open reading frame; Pax2, paired box gene 2; PREs, PAX2-response elements; PTIP, PAX-transactivation domain-interacting protein; SET1, Su(Var)3-9, E(z), Trx. PTIP targets HMTase complexes to gene promoters. PAX2 binds to PREs in the promoters of a range of genes that are required for the development of various organs. PTIP/Swift is brought to promoters by Pax2, and recruits SET1-like lysine methyltransferase complexes that methylate H3K4, thereby activating gene transcription. PTIP was found to be an integral component of several SET-domain-containing HMTase complexes that differ in their catalytic subunits, which can be MLL2 (Issaeva et al., 2007), MLL3 plus MLL4 (Cho et al., 2007) or MLL2 plus MLL3 (Patel et al., 2007). The differences in the catalytic components of the PTIP complexes identified in these studies might indicate that PTIP associates with a core subunit that is common to all MLL complexes, or might reflect the different HMTase complexes that exist in the various cell types used in these studies. H3K4, lysine 4 of histone H3; HMTase, histone methyltransferase; Me, methyl; MLL, mixed-lineage leukaemia; ORF, open reading frame; Pax2, paired box gene 2; PREs, Pax2-response elements; PTIP, Pax2-transactivation domain-interacting protein; SET, Su(Var)3-9, E(z), Trx.

PTIP and genome stability

Disruption of murine Ptip has shown that it has a crucial role in the preservation of genome stability. The homozygous disruption of Ptip in mice is lethal at embryonic day 9.5 (Cho et al., 2003). At embryonic day 7.5, the cells have entered S phase and are replicating DNA although a high number of DSBs can be detected. These DSBs do not appear to be a consequence of apoptosis because the nuclei are not pyknotic (Cho et al., 2003). By embryonic day 8.5, the cells are arrested in G2 and contain a high number of DSBs, and they die before embryonic day 9.5. Therefore, chromosomes appear to fragment during DNA replication in Ptip−/− cells, causing G2 arrest and death (Cho et al., 2003). This phenotype is reminiscent of embryos from mice lacking the ATR protein kinase (Brown & Baltimore, 2000; de Klein et al., 2000). When replication forks stall at obstacles such as DNA damage, ATR stabilizes them and prevents their disassembly. PTIP might also be required to protect replication forks that stall, and the failure to stabilize stalled replication forks in its absence would cause S phase-specific DSBs (Cimprich & Cortez, 2008). Replication forks collapse, occasionally forming DSBs, which are rescued and repaired by homologous recombination (HR; Sancar et al., 2004). An interesting possibility is that PTIP could regulate HR, in which case the accumulation of DSBs in S phase observed in Ptip-null embryos might reflect collapsed forks that are not rescued by HR. It will be important to evaluate this possibility and whether PTIP is also required to prevent DSB formation during S phase in adult cells, as the appearance of high numbers of DSBs in PTIP-null embryos might reflect the rapid rates of embryonic cell division. In fact DSBs initially appear in the embryonic ectoderm, which seems to be particularly sensitive to DNA damage. It has yet to be established whether PTIP is required for S-phase progression in adult tissues; however, its localization at the sites of DNA replication indicates that PTIP might have an important role in S phase in adult cells (Issaeva et al., 2007).

Human cells that are depleted of PTIP, or cells from Ptip-null mice, are hypersensitive to ionizing radiation (IR), which causes DSBs (Cho et al., 2003; Gong et al., 2009; Jowsey et al., 2004). There
are two main mechanisms for the repair of DSBs: non-homologous end joining (NHEJ), which occurs primarily in G1 and involves the re-ligation of DNA ends; and HR, which is used primarily in S and G2 phases, and requires an intact sister chromatid or homologous chromosome to direct the repair of the DSB (Sonoda et al., 2006). HR is important not only for DSB repair but also for the rescue of blocked or collapsed replication forks. Therefore, it is tempting to speculate that the appearance of DSBs in Pitp-null embryos and the IR hypersensitivity of cells lacking PTIP could be explained by a common role for PTIP in regulating HR. It will be important to measure how well agents that cause DSBs or block replisomes can induce HR in cells lacking PTIP. PTIP interacts with the MRN complex and with the Bloom syndrome protein (Cho et al., 2007; Patel et al., 2007), both of which are known to be crucial for DNA-damage signalling and HR; however, the significance of these interactions is not yet clear.

The stalling of replisomes at DNA lesions leads to Lys 63-linked polyubiquitination of PCNA—a processivity factor for DNA polymerases—that facilitates the bypass of these lesions (Ullrich, 2005). The depletion of PTIP from Xenopus extracts or from human cells markedly reduces the level of PCNA ubiquitination induced by replication blockage and attenuates the recruitment of lesion-bypass factors to chromatin (Gohler et al., 2008). Could defective DNA-lesion bypass explain the high level of DSBs seen in PTIP-null embryos (Cho et al., 2007)? Defects in the early embryonic lethality in mice (Wittschieben et al., 2000); however, more work is needed to determine whether this is the cause of DSBs in embryos lacking PTIP. The molecular mechanisms whereby PTIP promotes PCNA ubiquitination are not yet understood, but could involve the recruitment of PCNA ubiquitin ligases to sites of blocked DNA replication. The identity of these ligases is not yet clear, although two candidates have been proposed (Motegi et al., 2008; Unk et al., 2008). However, given the connection between PTIP and chromatin modifiers, it is also possible that PTIP controls PCNA ubiquitination indirectly by creating a chromatin environment that favours the access of PCNA-modifying factors. An important question is therefore whether histone methylation influences PCNA modification.

PTIP and DNA-damage signalling

DNA damage poses a potentially serious threat to genome stability. DNA lesions activate the ATM and ATR protein kinases, which, in turn, activate the CHK1 and CHK2 kinases (Cimprich & Cortez, 2008). Together, these kinases orchestrate many protective cellular responses, including the slowing or arrest of cell-cycle progression, the activation of DNA repair and apoptosis (Sancar et al., 2004). ATM and ATR phosphorylate a wide range of substrates on pS/T-Q motifs (Sancar et al., 2004), although they require adaptor proteins—such as 53BP1—to enable the phosphorylation of different subsets of downstream substrates, such as the tumour suppressor p53, and the CHK1 and CHK2 kinases. 53BP1 is important not only to facilitate the phosphorylation of some substrates by ATM/ATR, but also for DNA repair and the activation of cell-cycle checkpoints (Zgheib et al., 2005).

PTIP seems to be an adaptor protein for ATM. In response to DSBs, ATM and CHK2 phosphorylate the p53 tumour suppressor at Ser 15 and Ser 20, respectively. Depletion of PTIP from human cells does not affect the DSB-induced stabilization of p53, but causes a reduction of the ATM-dependent phosphorylation of p53 at Ser 15 (Jowsey et al., 2004), which normally promotes p53-dependent transcription, thereby leading to the upregulation of proteins that block cell-cycle progression or cause apoptosis (Shiloh, 2006). Consequently, cells that lack PTIP have defects in the induction of p53-responsive genes such as p21 (Jowsey et al., 2004). How PTIP and the other adaptor proteins function at the molecular level to facilitate the phosphorylation of distinct subsets of ATM/ATR targets is an essential, but poorly understood, issue.

BRCT domains are small modular domains that are often, but not always, found in pairs in proteins that regulate responses to DNA damage, such as 53BP1, MDC1 and BRCA1 (Bork et al., 1997). Some BRCT domain pairs are able to bind to phosphorylated serine or threonine residues (Manke et al., 2003; Patel et al., 2004). PTIP has three pairs of BRCT domains: one at the amino terminal; DSB, DNA double-strand break; MLL, mixed-lineage leukaemia; PA1, PTIP-associated protein 1; 53BP1, p53-binding protein 1; pS-Q-V-F, phospho-Ser/Thr-Gln; PTIP, PAX-transactivation domain-interacting protein; Q, polyglutamine-tract region; Smad2, SMA- and MAD-related protein 2.
PTIP binds at or near sites of DNA damage

PTIP binds to sites of DNA damage (Issaeva et al., 2007; Jowsey et al., 2004; Manke et al., 2003); however, the underlying molecular mechanisms of its recruitment are only beginning to be understood. The histone variant H2AX is phosphorylated in response to DNA damage on Ser139, thereby allowing its direct interaction with a pair of BRCT domains in the adaptor protein MDC1 (Stucki et al., 2005). MDC1 subsequently recruits the MRN complex (Stucki et al., 2005) and the E3 ubiquitin ligase RNF8—which catalyses the ubiquitination of proteins at sites of DNA damage (Bin & Elledge, 2007; Huen et al., 2007; Kolas et al., 2007; Mailand et al., 2007). MDC1, RNF8 and the E2 ubiquitin-conjugating enzyme UBC13 are all required for the binding of 53BP1 to sites of DNA damage, possibly because 53BP1 binds to proteins that are ubiquitinated by UBC13–RNF8 at DSBs. UBC13-dependent and RNF8-dependent ubiquitination events have also recently been shown to be required for the formation of PTIP-IRIF after PTIP overexpression (Gong et al., 2009). Although RNF8 is also required for 53BP1 recruitment, and although PTIP and 53BP1 associate in an ATM-dependent manner after DNA damage, the recruitment of PTIP to IRIF is independent of 53BP1 (Gong et al., 2009; Jowsey et al., 2004). PTIP does not seem to be capable of binding to ubiquitin directly (Gong et al., 2009) and has no obvious ubiquitin binding motifs; therefore, it is possible that a PTIP-associated protein mediates the binding of PTIP to ubiquitinated proteins near the sites of DNA damage. This protein is unlikely to be a component of the MLL HMTag complexes, as these proteins do not form IRIF (Issaeva et al., 2007), and MLL3, for example, is not required for PTIP to bind at sites of DNA damage (Gong et al., 2009). By contrast, it is possible that UBC13-catalysed and RNF8-catalysed ubiquitination alters the activity of one or more proteins or enzymes at DSBs, which in turn leads to PTIP recruitment. In any case, the C-terminal BRCT domains of PTIP are required for its retention at DSBs (Fig 2). It is likely that the identification of ligands of these domains other than those already

The PA1–PTIP complex

Gel-filtration assays have recently revealed that there are two pools of PTIP in cells: a higher molecular weight pool that contains the known PTIP-associated HMTag complexes (Fig 1) and a smaller complex that also contains PA1. It is clear that PA1–PTIP is involved in DNA damage responses, but it is not yet clear whether PTIP-associated HMTag complexes participate in these responses. 53BP1 is part of a large (non-PTIP-containing) protein complex before DNA damage; after DNA damage, ATM-phosphorylated 53BP1 binds to PTIP, presumably to the pool associated with PA1. It is likely that PTIP also associates with other proteins in a phosphorylation-dependent manner after DNA damage. 53BP1, p53-binding protein 1; ATM, ataxia telangiectasia mutated; DBD, DNA double-strand break; HMTag, histone methyltransferase; MLL, mixed-lineage leukaemia; PA1, PTIP-associated protein 1; PAX, paired box; PRE, PAX2-response element; PTIP, PAX-transactivation domain-interacting protein. (Gong et al., 2009), suggesting that the interaction of PTIP with PA1 is crucial for the preservation of genome stability. PA1 depends on PTIP to form IRIF, whereas the binding of PTIP at or near DSBs does not require PA1 (Gong et al., 2009). Taken together, these data suggest that the pool of PTIP containing PA1 is important for DNA damage responses. The molecular mass of the PTIP–PA1 complex in non-irradiated cells is not much larger than the combined masses of PTIP and PA1, suggesting that there might be no other proteins in this complex. However, it is likely that extra proteins are recruited to the PTIP–PA1 complex after DNA damage in the same manner that 53BP1 binds to PTIP, and it would be interesting to perform gel-filtration experiments after the exposure of cells to IR in order to verify this possibility. In the gel-filtration experiments described above, 53BP1 eluted in a high-molecular weight complex (>2 MDa) of unknown composition, which probably contained USP28 (Zhang et al., 2006) and was distinct from the larger PTIP-containing complex. We predict that, after DNA damage, 53BP1 is phosphorylated by ATM and the entire 53BP1 complex becomes associated with the PTIP–PA1 complex. Therefore, identifying all the components of each complex is important to obtain a complete picture of how 53BP1 and PTIP act to protect genome stability. The consequences of the genetic ablation of PA1 are unknown and it will be important to see how closely the phenotype of PA1-null organisms recapitulates that of PTIP-null mice.

et al., 2003). Furthermore, BRCT pair C2 of PTIP does not seem to be sufficient to interact with the phospho-Ser25 of 53BP1 in vivo or in vitro. Instead, pairs C1 and C2 both seem to be required (Gong et al., 2009; Munoz et al., 2007). The need for two BRCT domain pairs in PTIP to bind to 53BP1 Ser25 is not yet understood, especially given the fact that pair C2 alone can bind to other pS/T-Q-V-F phosphoryleptides (Manke et al., 2003). In any case, the mutation of 53BP1 Ser25 causes defects in the DNA-damage response, as do mutations in the PTIP C-terminal BRCT domains that prevent its interaction with 53BP1 phospho-Ser25 (Munoz et al., 2007). Therefore, it is likely that the interaction between PTIP and 53BP1 phospho-Ser25 is an important, although poorly understood, event for an intact DNA-damage response. 53BP1 is important for cellular resistance to genotoxins and to DNA damage signaling (Gong et al., 2009), suggesting that the interaction of PTIP with PA1 is crucial for the preservation of genome stability. PA1 depends on PTIP to form IRIF, whereas the binding of PTIP at or near DSBs does not require PA1 (Gong et al., 2009). Taken together, these data suggest that the pool of PTIP containing PA1 is important for DNA damage responses. The molecular mass of the PTIP–PA1 complex in non-irradiated cells is not much larger than the combined masses of PTIP and PA1, suggesting that there might be no other proteins in this complex. However, it is likely that extra proteins are recruited to the PTIP–PA1 complex after DNA damage in the same manner that 53BP1 binds to PTIP, and it would be interesting to perform gel-filtration experiments after the exposure of cells to IR in order to verify this possibility. In the gel-filtration experiments described above, 53BP1 eluted in a high-molecular weight complex (>2 MDa) of unknown composition, which probably contained USP28 (Zhang et al., 2006) and was distinct from the larger PTIP-containing complex. We predict that, after DNA damage, 53BP1 is phosphorylated by ATM and the entire 53BP1 complex becomes associated with the PTIP–PA1 complex. Therefore, identifying all the components of each complex is important to obtain a complete picture of how 53BP1 and PTIP act to protect genome stability. The consequences of the genetic ablation of PA1 are unknown and it will be important to see how closely the phenotype of PA1-null organisms recapitulates that of PTIP-null mice.

The PA1–PTIP complex

Gel-filtration assays have recently revealed that there are two pools of PTIP in cells (Cho et al., 2007; Gong et al., 2009): a higher molecular weight pool (of 2 MDa) containing the HMTag complexes, and a lower molecular weight pool (of ~200 kDa) that contains the newly identified protein PA1 (Fig 3). PA1 associates directly with PTIP (Cho et al., 2007) and, importantly, depletion of PA1 renders cells hypersensitive to IR (Gong et al., 2009), as is the case for PTIP. The second BRCT domain of the N1 pair of PTIP is required for its interaction with PA1 (Fig 2) and a mutant form of PTIP lacking this domain cannot rescue the IR hypersensitivity of PTIP-null cells
known—that is, 53BP1, MLL2 and Smad2—will be important for resolving this issue.

**PTIP in lower eukaryotes?**

Esc4 is a multi-BRCT domain-containing protein from *Saccharomyces cerevisiae* with a relatively high degree of sequence similarity to PTIP (Rouse, 2004)—especially in the BRCT domains—that was implicated in the response to DNA-damaging agents in several yeast genetic screens (Chang et al, 2002; Hanway et al, 2002). Esc4 is required to overcome blocks to DNA replication and is an important target of Mec1, the yeast orthologue of ATR kinase (Roberts et al, 2006; Rouse, 2004; Zappulla et al, 2006). Although Esc4 and PTIP are both required to preserve genome stability during S phase (Cho et al, 2004; Zappulla et al, 2006), there are some notable differences between the two proteins: PTIP has one pair of BRCT domains at the N terminus and two at the C terminus, whereas Esc4 has two pairs at the N terminus and one at the C terminus; PTIP is essential in mice, whereas Esc4 is not essential in yeast (Chang et al, 2002); PTIP is required for cellular responses to DSBs, but Esc4 is not (Roberts et al, 2006; Rouse, 2004; Zappulla et al, 2006); and none of the reported Esc4-interacting proteins are HMTases (Chin et al, 2006). Therefore, in functional terms, Esc4 and PTIP may have more differences than similarities and it is unclear whether PTIP is a genuine orthologue of Esc4 that has evolved additional functions in higher eukaryotes or whether the sequence similarity between Esc4 and PTIP is simply due to the conservation of the BRCT domains.

**Dual roles for PTIP?**

It is clear that PTIP controls transcription by targeting HMTases to gene promoters, and this function is relatively well understood in mechanistic terms (Fig 1). PTIP also controls aspects of the cellular response to DNA damage, although our understanding of this process is still at an early stage. Are these roles separate or related? H3K4 methylation is known to be important for the maintenance of genome stability. For example, the PHD domain of the ING2 tumour suppressor—a subunit of the HDAC1 histone deacetylase complex—binds to methylated H3K4 after DNA damage. This stabilizes the association of the HDAC1 complex with promoters that are repressed in response to DNA damage (Shi et al, 2006). Chd1, which is a protein that also binds to methylated H3K4, regulates S-phase progression in yeast (Biswas et al, 2008). In addition, the ING1 tumour suppressor must also bind to methylated H3K4 to promote DNA repair and/or apoptosis after DNA damage (Pena et al, 2008). Therefore, defects in H3K4 methylation could, in principle, contribute to the formation of the S-phase DSBs observed in PTIP-null embryos. The methylation of histone H3 at other lysine residues is also important for DNA-damage responses, as it promotes the assembly of signalling complexes at sites of DNA damage. For example, the methylation of H3K79 by DOT1L enables the binding of the Tudor domains of 53BP1, which contributes to 53BP1 recruitment to DSBs (Huyen et al, 2004)—a process in which the methylation of H4K20 also has a role (Botuyan et al, 2006). PR-Set7/SET8 methylates H4K20, localizes at sites of DNA replication (Tardat et al, 2007) and its depletion causes defects that are notably similar to those seen in cells lacking PTIP, such as the induction of DSBs in S phase and an accumulation of cells in G2 (Houston et al, 2008; Jørgensen et al, 2007; Tardat et al, 2007). This phenotype is notably reminiscent of the phenotype of PTIP-null embryos (Cho et al, 2003) and it is tantalizing to speculate that PTIP regulates H4K20 methylation in some manner. However, there is currently no evidence that PTIP regulates H4 methylation and immunoprecipitates of cells in which PTIP has been overexpressed contain no detectable H4 HMTase activity (Cho et al, 2007).

Histone methylation is important for DNA-damage responses, but are the PTIP-associated HMTases involved? Although PTIP and PA1 form IRIF, the components of the MLL HMTase complexes do not (Gong et al, 2009; Issaeva et al, 2007), and the knockdown of MLL3 does not affect PTIP IRIF (Gong et al, 2009). Therefore, it has been argued that this type of complex does not have a role in preserving genome integrity but rather regulates transcription. Our impression is that this is the case, although excluding a role for PTIP HMTases in DNA-damage responses based on IRIF analysis might not be valid without testing the effect that depleting MLL HMTases has on cellular sensitivity to DNA damage. Not all proteins that are involved in DNA-damage responses form foci and, even when they do, focus formation is not always functionally important (Ball et al, 2005). Therefore, it will also be important to address definitively whether the PTIP-associated HMTases are required for a DNA-damage response. In any case, the most important task is to determine the full composition and mode of operation of the PTIP complexes that are known to be DNA-damage responsive before and after DNA damage (Fig 3).

**Conclusion and future challenges**

There are at least two PTIP-associated complexes that most of the available data suggest are functionally distinct (Fig 3), although further experiments are needed to confirm this. We need to know what controls the distribution of PTIP among these complexes. Does the depletion of MLL2/MLL3/MLL4 HMTases cause genotoxic hypersensitivity, or defects in DNA repair or signalling? By what mechanism does the PTIP–PA1 complex promote cell survival after DNA damage? Are there other proteins apart from 53BP1 that associate with PTIP exclusively after DNA damage or in S phase? PTIP is recruited by transcription factors to gene promoters, where it recruits H3K4 methyltransferases to modulate gene expression. It also binds to sites of DNA damage in a manner that seems to require protein ubiquitination. How is this achieved? Does PTIP regulate HR? Why does PTIP interact with 53BP1? Clearly, much more information is needed to understand how PTIP regulates genome stability. It will be fundamentally important to fill in these gaps and to reconcile the functions of PTIP in histone modification, transcription and DNA damage (Sidebar A).

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**Sidebar A | In need of answers**

1. Are the transcriptional and DNA-damage associated functions of PTIP mechanistically separate?
2. Why is protein ubiquitination required for the recruitment of PTIP to sites of DNA damage?
3. What are the molecular mechanisms whereby PTIP and PTIP–PA1 control genome stability and resistance to DNA damage?
4. How does PTIP promote PCNA ubiquitination?
5. What is the role of PA1 at the molecular level?
6. How is PTIP distributed among several different complexes?
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REFERENCES


PTIP, histone methylation and genome stability

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