# Trithorax group proteins: switching genes on and keeping them active

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Abstract | Cellular memory is provided by two counteracting groups of chromatin proteins termed Trithorax group (TrxG) and Polycomb group (PcG) proteins. TrxG proteins activate transcription and are perhaps best known because of the involvement of the TrxG protein MLL in leukaemia. However, in terms of molecular analysis, they have lived in the shadow of their more famous counterparts, the PcG proteins. Recent advances have improved our understanding of TrxG protein function and demonstrated that the heterogeneous group of TrxG proteins is of critical importance in the epigenetic regulation of the cell cycle, senescence, DNA damage and stem cell biology.

Epigenetic
A form of gene expression maintenance in which the heritable state of gene activity neither requires the continuous presence of the initiating signal nor involves changes in the DNA sequence.

Homeobox genes
(HOX genes). A family of genes that encode transcription factors which are essential for patterning along the anterior–posterior body axis.

Homeotic transformations
The consequences of mutations that lead to the transformation of the identity of one body segment into the identity of another.

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doi:10.1038/nrm3230

Most of the cells in multicellular organisms contain the same genetic information, but differential spatio-temporal gene regulation gives each cell its own fate and identity. Therefore, cell fate choices require the establishment of specific gene expression patterns, which then will determine a specific cell lineage. A cellular memory system is needed to stably transmit these gene expression patterns during cell division and replication. This genome regulation is essential for homeostasis in multicellular organisms and is mainly achieved by epigenetic mechanisms regulating chromatin structure.

For instance, the regulation of the expression patterns of Homeobox genes (HOX genes) in *Drosophila melanogaster* is established during early embryogenesis by a cascade of maternal and zygotic transcription factors (for a review, see REF. 1). These early acting factors disappear at later developmental stages, and the memory of the transcriptional state of HOX genes is then maintained by two groups of proteins: Trithorax group (TrxG) proteins, which activate transcription, and Polycomb group (PcG) proteins, which repress transcription.

PcG genes were identified from mutations that induce HOX genes outside their usual expression domains, causing homeotic transformations[2]. A few years later, the founding member of the TrxG, TRX, was characterized as a positive regulator of HOX genes[3,4]. Interestingly, TRX function was not needed to initiate HOX gene expression, but rather to maintain it in the appropriate body segments later in development. This finding was at the origin of the idea that, as in the case of PcG genes, *Trx* may be involved in the epigenetic inheritance of HOX gene expression states[3,5]. Other TrxG proteins have been subsequently identified based on mutant phenotypes that reflect the loss of function of HOX genes, or by genetic screens for suppressors of PcG-dependent mutant phenotypes[6], leading to the definition of TrxG proteins as antagonists of PcG-dependent silencing (BOX 1).

PcG proteins regulate many genes in addition to HOX genes (reviewed in REF. 7), and the same is true for TrxG proteins, which, through their roles in methylating histones and remodelling chromatin, are involved in the maintenance of active chromatin states (BOX 2), tumorigenesis and embryonic stem (ES) cell self-renewal[8], cell fate choice and proliferation[9]. TrxG proteins have also been implicated in X-chromosome inactivation[10], apoptosis[11], cell cycle regulation[12], growth plasticity and regeneration, as well as in the stress response, during which they activate senescence and DNA damage checkpoints[13,14]. However, it is unclear whether they regulate these cellular processes mainly by antagonizing PcG function or by globally activating gene expression (BOX 1). Indeed, TrxG proteins can play widespread parts in general transcriptional activity[15,16] and two TrxG genes have been shown to encode for subunits of the *D. melanogaster* Mediator complex, which is involved in general transcriptional activation[17]. In this Review, we describe TrxG complexes and their enzymatic functions, and evaluate recent data aimed at understanding how they are recruited to their targets. Furthermore, we discuss the molecular mechanisms used by TrxG complexes to regulate genome function.



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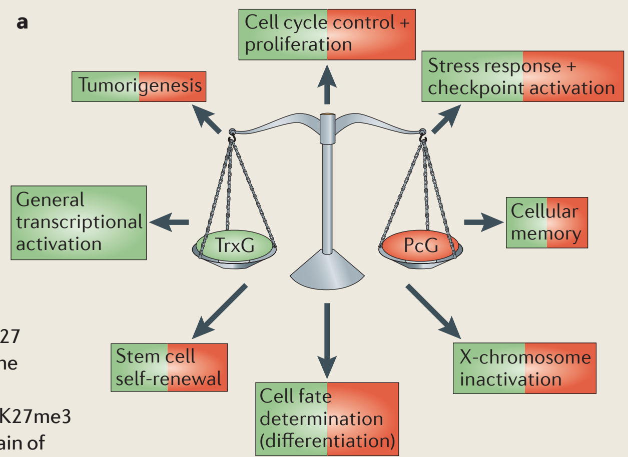
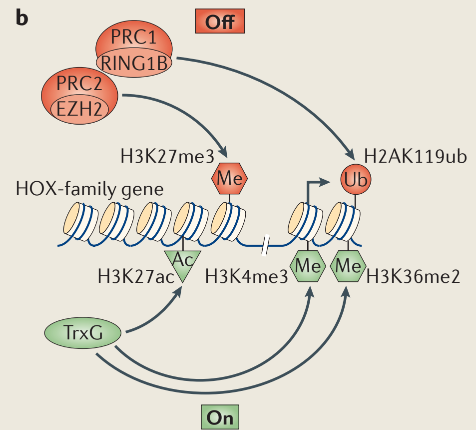
Box 1 | **The molecular function of PcG complexes and the antagonistic action of TrxG proteins**

Polycomb group (PcG) proteins, which have functions that are generally antagonistic to Trithorax group (TrxG) proteins, play a pivotal part in the dynamic regulation of many key developmental genes to define cellular identities and regulate key biological processes (see the figure, part **a**). Biochemical and genetic studies demonstrated that PcG silencing in *Drosophila melanogaster* and vertebrates involves the activity of multiprotein complexes (reviewed in REF. 7). Among these, Pc repressive complex 2 (PRC2) has histone methyltransferase activity that is specific for the trimethylation of Lys27 on histone H3 (H3K27me3), which is catalysed by the SET domain-containing protein Enhancer of zeste homologue 2 (EZH2; see the figure, part **b**). The H3K27me3 mark is specifically recognized by the chromodomain of Pc, a subunit of PRC1-type complexes. These complexes can monoubiquitylate Lys119 on histone H2A (H2AK119ub), a process which is catalysed by RING finger protein 1B (RING1B) and contributes to gene silencing. In addition to these two major PcG complexes, several additional PcG complexes with different enzymatic activities contribute to the plethora of biological processes that are regulated by PcG proteins, including cell cycle control, proliferation and cellular senescence, X-chromosome inactivation, stem cell plasticity and cell fate determination, and cancer (reviewed in REF. 7).

PcG complexes are recruited to DNA via specific DNA elements, termed PcG response elements (PREs), and can use multiple mechanisms to repress target genes involved in a wide range of cellular processes (reviewed in REF. 185). PRC2-mediated methylation on H3K27 might directly interfere with transcriptional activation by blocking the deposition of active histone marks (reviewed in REF. 185). PRC1-mediated ubiquitylation on H2A, which has been suggested to interfere with transcriptional elongation and the binding of the PRC1 complex to chromatin, can induce chromatin compaction, interfering with nucleosome-remodelling activities and RNA polymerase II recruitment (reviewed in REF. 186). PcG-mediated repression can be dynamic and is specifically counteracted by TrxG proteins via the histone modifications that they mediate, including acetylation of H3K27 (H3K27ac), H3K4me3, dimethylation of Lys36 on histone H3 (H3K36me2) and/or nucleosome-remodelling activities. However, TrxG proteins have been also identified as part of the general transcription machinery, indicating that their antagonistic action towards PcG proteins is only one of their functions.




## Diversity of TrxG proteins

TrxG proteins are evolutionarily conserved chromatin regulators that can be divided into three classes based on their molecular function (FIG. 1a). One class includes SET domain-containing factors that can methylate histone tails (for a review, see REF. 18). The second class contains ATP-dependent chromatin-remodelling factors, including proteins that can 'read' the histone methylation marks that are laid down by the SET domain proteins. The third class includes TrxG proteins that can directly bind specific DNA sequences and is comprised of some histone modifiers and chromatin remodellers, as well as proteins that are not categorized within the first two classes. Most of these TrxG proteins exert their function as part of large multi-protein complexes that have either histone-modifying or nucleosome-remodelling activities which cooperate to regulate gene expression.

*Histone-modifying complexes.* Trimethylation of Lys4 on histone H3 (H3K4me3) is a hallmark of active genes. Yeast SET domain-containing 1 (Set1) was the first histone methyltransferase (HMT) shown to specifically catalyse the mono-, di- and trimethylation of H3K4 within a protein complex called COMPASS (complex proteins associated with Set1). Mammalian homologues of Set1 were then found to form COMPASS-like complexes (FIG. 1b). The first mammalian COMPASS-like component to be identified was MLL1 (also known as HRX and ALL-1), which was originally discovered as the gene inducing human leukaemia caused by chromosome band 11q23 translocations[19,20]. Multiple MLL homologues exist in plants and animals, forming different COMPASS-like complexes that provide gene-specific regulation (FIG. 1b). In mammals, six COMPASS-related complexes with essential and non-redundant functions have been identified that can methylate H3K4

SET domain
(Su(var)3-9, Enhancer of Zeste, Trithorax). A motif ~130 amino acids in length that provides histone methyltransferase activity. It is found in many chromatin-associated proteins, including some Trithorax group and Polycomb group proteins.



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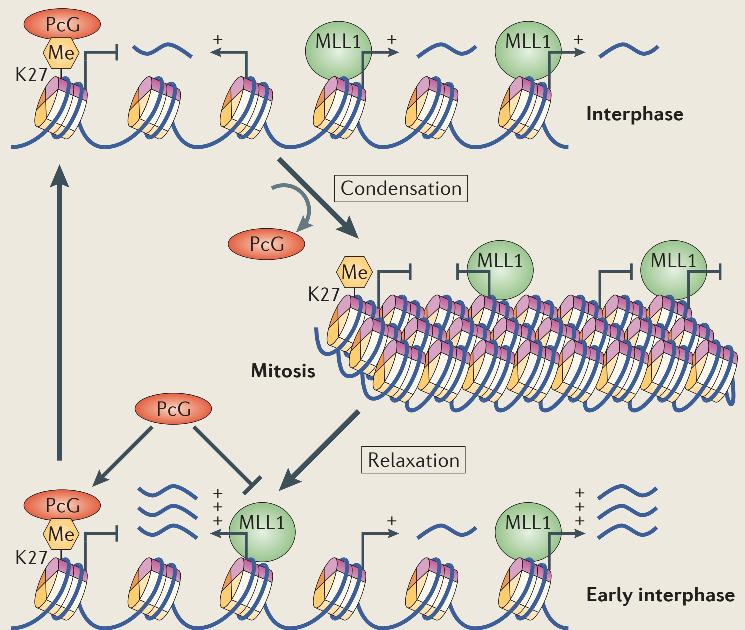
Box 2 | **Epigenetic inheritance of active chromatin states**




The key question in the epigenetic inheritance of gene regulation is how chromatin signatures are stably transmitted during cell division. Surprisingly, a recent report revealed that histones at Trithorax group (TrxG) and Polycomb group (PcG) binding sites are exchanged multiple times during cell division[187]. Thus, histone modifications are erased and re-established several times during a cell cycle, questioning the ability of sharply localized histone marks to transmit epigenetic information.

In the case of PcG-mediated silencing, it has nevertheless been proposed that a self-perpetuating cycle of trimethylation of Lys27 on histone H3 (H3K27me3) performed by Pc repressive complex 2 (PRC2) is responsible for the propagation of silent chromatin marks. This involves the binding of PRC2 to its own H3K27me3 mark[188,189], by virtue of which PRC2 may be able to bind back onto the chromatin template upon transient loss that might occur during DNA replication or mitosis (see the figure). This seems a plausible hypothesis, as PcG-associated histone marks are distributed over large chromatin domains, facilitating the re-establishment of these repressive histone marks.

By contrast, H3K4me3 is more sharply localized to promoter regions, making it unlikely that the histone mark alone can provide epigenetic inheritance. In addition, mutation of the histone methyltransferase (HMT) activity of MLL in mice results in only mild phenotypes, whereas MLL-null mice die during early embryogenesis, indicating a HMT-independent function to propagate active chromatin states[24,190].

During mitotic chromosome condensation, gene expression is globally shut off, and most transcription factors dissociate from mitotic chromatin. Interestingly, unlike other HMTs (or PcG proteins), MLL1 remains associated with condensed mitotic chromosomes (see the figure)[191]. Genome-wide mapping of MLL1 in mitotic and interphase cells revealed that MLL1 binding sites change throughout the cell cycle. Some target sites are kept and others are lost during mitosis, whereas a subset are exclusively occupied in mitosis — these sites correspond to genes that are highly expressed in the subsequent interphase. By contrast, genes that are uniquely bound by MLL1 in interphase exhibit only moderate expression levels. Mitotic retention of MLL1 may thus serve as a 'bookmark' that accelerates the kinetics of gene reactivation after mitotic exit[191]. The precise mechanism for this mitotic bookmarking function remains unknown, but seems to be largely independent of H3K4 trimethylation. The fate of TrxG proteins during DNA replication, the other phase of the cell cycle in which erasure of chromatin-mediated memory is most likely to occur, is also unknown. Research in both areas will be critical for the understanding of epigenetic inheritance in eukaryotes.

through their conserved SET domain (FIG. 1b). SET1A- or SET1B-containing complexes are the ones most closely related to yeast COMPASS and include the unique WD repeat-containing 82 (WDR82) subunit. SET1-containing COMPASS complexes mediate the bulk of H3K4 trimethylation in mammalian cells[21] and *D. melanogaster* cells, indicating that they are involved in global gene activation.

COMPASS-like complexes containing MLL1 or MLL2 include the unique subunit menin (FIG. 1b), which can act as a tumour suppressor[22] and is required for localization of the complex to chromatin[23]. MLL1 is required for H3K4 trimethylation at a small subset of mammalian genes, including HOX genes[21], whereas menin is required for essentially all H3K4 trimethylation at HOX genes. In addition, knockout of *Mll1* and *Mll2* in mice confirmed that the proteins encoded by these genes have a non-redundant function[24,25]. Furthermore, MLL1 has been purified with MOF, a MYST family histone acetyltransferase (HAT) that specifically acetylates H4K16, linking histone methylation and acetylation to gene activation[26].

MLL3 and MLL4-containing complexes are important for H3K4 methylation at retinoic acid target genes but are dispensable for H3K4 methylation at HOX loci[27,28]. They also contain the demethylase UTX (also known as KDM6A) (FIG. 1b), which can remove PcG-mediated repressive H3K27 methylation. These complexes might thus relieve PcG-mediated repression in addition to sustaining transcription via their H3K4 methyltransferase activity.

Homologues of mammalian MLL proteins have been characterized in *Arabidopsis thaliana*, *Caenorhabditis elegans* and flies, indicating that their function is widely conserved in plants and animals[29–31]. *C. elegans* contains one SET1 protein, SET-2, and one MLL-like protein, SET-16, which is most similar to MLL3 and MLL4 in

MYST
A family of histone acetyltransferases that is defined by the founding members Moz, Ybf2 (Sas3), Sas2 and Tip60.



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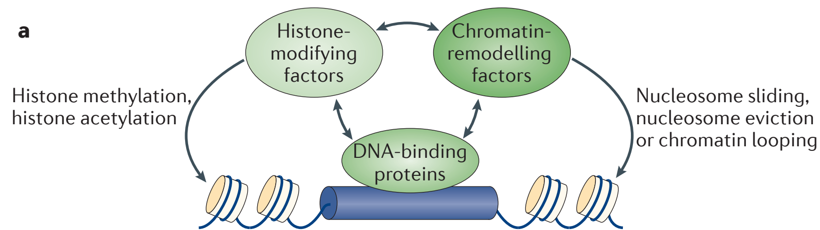
**a**




**b**

| Complex | Comment | Mammals | *D. melanogaster* | *C.elegans* | Plants |
|---|---|---|---|---|---|
| ***Histone-modifying complexes*** | | | | | |
| COMPASS | Global gene activation; mediates bulk trimethylation of K4 on histone H3 (H3K4me3) | SET1A,B; CXXC1, WDR82; ASH2L, DPY30, HCF1, RBBP5, WDR5 | SET1; CXXC1, WDR82; ASH2, DPY30, HCF1, RBBP5, WD5 | SET-2 | ATX1–5; ASH2R, RBL, WDR5A |
| COMPASS-like | HOX-family gene regulation, tumour suppressor activity and HAT activity for H4K16 | MLL1,2, MOF; Menin; ASH2L, DPY30, HCF1, RBBP5, WDR5 | TRX; Menin; ASH2, DPY30, HCF1, RBBP5, WD5 | SET-16, UTX-1; ASH-2, DPY-30, WDR-5 | |
| | Activates nuclear receptors in mammals and ectysone-induced promoters in *D. melanogaster*; demethylase activity for H3K27me3 plays a part in dosage compensation and the attenuation of RAS signalling in *C. elegans* | MLL3,4, UTX; NCOA6, PA1, PTIP; ASH2L, DPY30, RBBP5, WDR5 | TRR, UTX; NCOA6, PA1; PTIP; ASH2, DPY30, RBBP5, WD5 | | |
| TAC1 | Counteracts PcG silencing, but also has a more global role in gene activation; has HAT activity for H3K27 in *D. melanogaster* | | TRX, CBP; SBF1 | | |
| ASH1 | Counteracts PcG silencing and has HMT activity for H3K36 and HAT activity for H3K27 in *D. melanogaster* | ASH1L | ASH1, CBP | | |
| ***ATP-dependent chromatin-remodelling complexes*** | | | | | |
| SWI/SNF | Binds acetylated histones via its bromodomain, regulates cell cycle, cell signalling, proliferation and chromosome segregation | BRM, BRG1; Actin, BAF45A, BAF45B, BAF45C, BAF45D, BAF47, BAF53A, BAF53B, BAF60A, BAF60B, BAF60C, BAF155, BAF170, BAF180, BAF200, BAF250A, BAF250B, BAP57, BRD7, BRD9 | BRD; Actin, BAF60, BAF180, BAP55, BAP111, Dalao, BAP170, OSA, MOR, SAYP, SNR1 | | |
| ISWI | Binds unmodified histones via its SANT domain and reads H3K4me3 via its PHD finger; regulates transcription, cell fate determination and differentiation | BPTF; RBAP46, RBAP48, SNF2L | NURF301; ISWI, NURF38, RBAP46, RBAP48 | NURF; ISW-1 | CHR11,17 |
| CHD1, CHD2 | Bind H3K4me3 via their chromodomains; have roles in development and ES cell maintenance; H3.3 deposition in *D. melanogaster* | CHD1,2 | CHD1,2 | | |
| CHD3, CHD4 | HDAC activity; regulate transcription, replication and DNA repair; regulate cell fate determination in *C. elegans* and counteract PcG silencing in plants | CHD3,4, HDAC1,2; RBAP46, RBAP48, MBD2, MBD3, MTA1, MTA2, MTA3, p66 | Mi2, RPD3; MBD2, MBD3, MTA, p55, p66, p68 | HDA-1; LET-418; MEP-1 | PKL |
| CHD6, CHD7, CHD8 | Bind dimethylated H3K4 (H3K4me2) and H3K4me3 via their chromodomains; counteract PcG silencing and regulate transcriptional elongation in *D. melanogaster* | CHD8; ASH2L, RBBP5, WDR5 | CHD6, CHD7; CHD8; KIS-L | | |



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◀ Figure 1 | **Classes of TrxG proteins and TrxG-containing complexes across species.** **a** | Trithorax group (TrxG) proteins bind to DNA in a sequence-specific manner, or act as large multimeric complexes that modify histones or contain ATP-dependent nucleosome-remodelling activity. DNA-binding proteins help to recruit chromatin-remodelling and histone-modifying complexes to regulate transcription, and chromatin-remodelling factors can 'read' the histone methylation marks that are laid down by histone-modifying complexes. **b** | Only TrxG members that have been biochemically characterized by the isolation of their multiprotein complexes are listed here. Components with enzymatic activities are shown in dark blue circles. Unique subunits in COMPASS (complex proteins associated with Set1) and COMPASS-like complexes are shown in light blue circles. Note that SWI/SNF (switch/sucrose nonfermentable) is a family of complexes, including *Drosophila melanogaster* BAP and PBAP and human BRG1-associated factor (BAF) and PBAF as well as embryonic stem (ES) BAF (esBAF). For simplicity, only the BAP and BAF complexes are shown here. The comment on each TrxG group member refers to mammals, if not otherwise specified. ASH, Absent, small and homeotic discs; ASH1L, ASH1-like; BPTF, bromodomain and PHD finger-containing transcription factor; BRD, Bromodomain-containing; BRM, Brahma; *C. elegans*, *Caenorhabditis elegans*; CBP, cyclic AMP response element-binding protein; CHD, chromodomain helicase DNA-binding; DPY, dumpy; HAT, histone acetyltransferase; HCF1, host cell factor 1; HDAC, histone deacetylase; HMT, histone methyltransferase; HOX, homeobox; ISWI, imitation switch; KIS-L, Kismet long; MBD, methyl-CpG-binding domain; MEP-1, MOG-interacting and ectopic P-granules 1; LET-418, lethal 418; MOR, Moira; NCOA6, nuclear receptor co-activator 6; NURF, nucleosome-remodelling factor; PA1, PTIP-associated 1; PcG, Polycomb group; PHD, plant homeodomain; PKL, PICKLE; PTIP, PAX transactivation activation domain-interacting protein; RBAP, retinoblastoma-binding protein; RBBP5, retinoblastoma-binding protein 5; SAYP, Supporter of activation of yellow protein; SET, SET domain-containing; SNR1, Snf5-related 1; TRR, TRX-related; WDR, WD repeat-containing.

RAS signalling
An intracellular signal transduction pathway involving RAS. RAS activates many signalling cascades involved in multiple developmental events controlling cell proliferation, migration and survival.

SWI/SNF
(Switch/sucrose nonfermentable). A chromatin-remodelling complex family that was first identified genetically in yeast as a group of genes required for mating type switching and growth on alternative sugar sources to sucrose. This complex is required for the transcriptional activation of ~7% of the genome.

Bromodomain
A conserved protein module, which was first identified in the *Drosophila melanogaster* protein Brahma and has subsequently been found in many chromatin-associated proteins. This domain can recognize acetyl-Lys motifs.

SANT domain
A conserved histone-binding domain that takes its name from the proteins in which it was initially identified: Swi3, ADA2, N-CoR and TFIIB.

mammals. Both SET-2 and SET-16 are required for global H3K4 methylation, but SET-2 seems to have a dominant role[14,32,33]. The *C. elegans* COMPASS-like complex includes SET-16, the demethylase UTX-1, which attenuates RAS signalling, and the dumpy 30 (DPY-30) subunit, which functions in dosage compensation[34]. *D. melanogaster* possesses three COMPASS-related complexes, which contain the SET domain-containing proteins SET1, TRX and TRX-related (TRR)[35]. In addition, the TAC1 complex contains TRX, the *D. melanogaster* homologue of MLL, and the HAT cyclic AMP response element-binding protein (CBP), which can acetylate H3K27 for gene activation[36–38].

An additional TrxG protein containing a SET-domain in *D. melanogaster* is Absent small and homeotic discs 1 (ASH1), which was shown to associate with CBP[39] and which may also be involved in the methylation of H3K36 (REF. 40). Thus, analogous to the specification of the mammalian TrxG MLL gene family, SET domain-containing proteins have diverged developmental functions in *D. melanogaster*.

***ATP-dependent chromatin-remodelling complexes.*** ATP-dependent chromatin-remodelling complexes open up chromatin by inducing nucleosome sliding or eviction, as well as by mediating chromatin looping. They are widely conserved and can be subdivided into different families on the basis of the sequence and structure of the ATPase subunit. The yeast SWI/SNF (switch/sucrose nonfermentable) subfamily was the first chromatin-remodelling complex to be discovered[41]. The TrxG member Brahma (BRM) is a bromodomain-containing protein in *D. melanogaster*, homologous to yeast SWI/SNF and mammalian BRM and BRG1 (also known as SMARCA4), that was identified in screens for suppressors of PcG-mediated homeotic transformations[6]. SWI/SNF complexes are variable and non-redundant, and they regulate the chromatin structure of a large number of genes implicated in the cell cycle, cell signalling, proliferation and chromosome segregation (reviewed in REF. 42).

The imitation switch (ISWI) family contains a unique SANT domain. *D. melanogaster* has a single ISWI ATPase, which forms the nucleosome-remodelling factor (NURF) complex (reviewed in REF. 42). The chromodomain helicase DNA-binding (CHD) family of chromatin modifiers is defined by the presence of chromodomains and can be divided into three subfamilies. CHD1 and CHD2 form the first subfamily and have important roles during development and ES cell maintenance[43]. CHD3 and CHD4 make up the second subfamily and are part of the Mi2–NuRD (Mi2–nucleosome remodelling and deacetylase) complex, which couples chromatin remodelling and histone deacetylation to mediate repressive functions involved in transcriptional regulation, replication, DNA repair and cell fate determination[44,45]. However, the plant CHD3 homologue PICKLE (PKL) counteracts PcG-mediated repression to regulate meristem activity in the *A. thaliana* root[46]. The third subfamily contains CHD6, CHD7 and CHD8 and includes the single *D. melanogaster* TrxG protein Kismet long (KIS-L), which activates transcription and counteracts PcG-dependent silencing by guiding ASH1 and TRX to their targets[15].

***TrxG proteins and sequence-specific DNA binding.*** In flies, TrxG complexes bind to DNA elements called TrxG response elements (TREs). These elements often coincide with PcG response elements (PREs), and an overlapping set of sequence-specific DNA-binding proteins (for example, the DNA-binding proteins Dorsal switch protein 1 (DSP1), GAGA factor (GAF) and Zeste) are involved in the recruitment of *D. melanogaster* PcG and TrxG complexes (reviewed in REF. 7). TRX has been shown to be recruited to its target genes independently of their activation status, whereas ASH1 is targeted to genes in an activation-dependent manner, thereby facilitating transcriptional elongation[47]. Similarly, BRM was shown to be recruited to polytene chromosomes upon activation of a TRE-containing transgene[48].

No mammalian TRE sequences have been identified so far. In mammals, MLL1 and MLL2 have a CXXC domain, a type of zinc-finger that can recognize unmethylated CpG sequences and is absent in the *D. melanogaster* homologue TRX. CpG-rich sequences (CpG islands) have been suggested to recruit PcG complexes in mammals[49], and they may also recruit TrxG complexes (FIG. 2a). Indeed, they recruit CXXC1 (also known as CFP1), a component of mammalian COMPASS[50] (FIG. 1b). Other DNA-binding proteins, such as nuclear factor Y (NF-Y) and NF-E2, were shown to interact with ASH2L, inducing its recruitment and mediating H3K4 trimethylation at target promoters[51,52]. Furthermore, ASH2-like (ASH2L) itself binds CG-rich DNA motifs, reinforcing the tethering of MLL complexes to their target chromatin[53,54].



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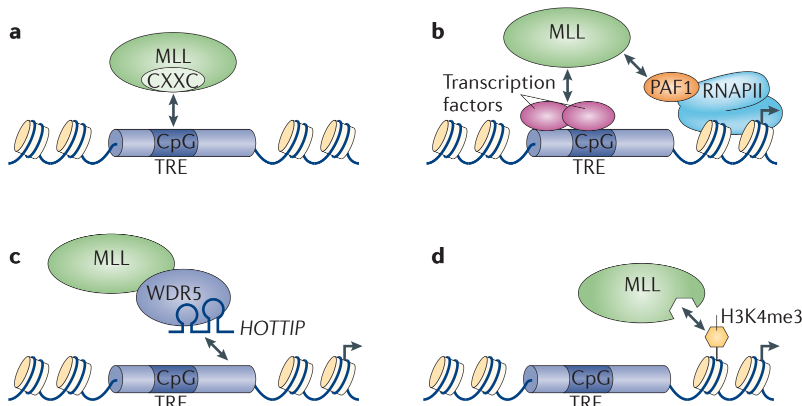


Figure 2 | **Multiple mechanisms recruit TrxG complexes to their target sites.** **a** | MLL can directly interact with CpG-rich sequences via its CXXC domain. **b** | Alternatively, MLL can be targeted to DNA by interacting with sequence-specific transcription factors or the polymerase-associated factor 1 (PAF1) elongation complex. Similarly, in flies, Trithorax group (TrxG) complexes can be recruited to TrxG response elements (TREs) via their interaction with sequence-specific DNA-binding proteins. Note that TREs have not been identified in mammals so far. **c** | RNAs, such as *HOXA* transcript at the distal tip (*HOTTIP*), can recruit MLL via their interaction with the adaptor protein WD repeat-containing 5 (WDR5). **d** | The plant homeodomain (PHD) finger domain of MLL can bind to trimethylated Lys4 on histone H3 (H3K4me3), helping to target MLL to chromatin. RNAPII, RNA polymerase II.

Nucleosome-remodelling factor
(NURF). A chromatin-remodelling complex identified in *Drosophila melanogaster* and belonging to the imitation switch subfamily.

Chromodomain
A motif of ~60 amino acids that is found in many chromatin-associated proteins and forms a binding pocket for methylated histone residues.

Bivalent chromatin domains
Domains that are characterized by the juxtaposition of active and inactive epigenetic histone marks.

Plant homeodomain finger
(PHD finger). A PHD-linked zinc-finger that chelates double zinc ions. This protein motif is found in many chromatin regulators and binds histones in a methylation-dependent or -independent manner.

*Trans* histone code
This term describes the fact that post-translational modifications on one histone tail can influence those on another, even when they are located on different histones, resulting in a specific gene expression output.

Recently, bioinformatic analysis has shown that MYC binding motifs colocalize with Pc repressive complex 2 (PRC2) components and the repressive H3K27me3 mark[55]. In mouse ES cells, MYC binds to and regulates loci previously identified as PcG targets, including genes that contain bivalent chromatin domains[56]. Therefore, MYC may directly recruit PcG and TrxG complexes to genes controlling growth and proliferation (see below). TrxG complexes can also be targeted via interaction with the polymerase-associated factor 1 (PAF1) elongating complex. PAF1 directly interacts with sequences flanking the CXXC domain of MLL proteins[57], and functions as a binding platform for MLL- and TRX-containing complexes to elongating RNA polymerase II[58,59] (FIG. 2b).

## Non-coding RNAs and TrxG recruitment

In addition to directly binding specific DNA sequences and interacting with other transcription factors, TrxG proteins can be recruited to their targets via their interaction with non-coding RNAs (ncRNAs). ncRNAs play a central part in gene-silencing mechanisms, whereas their role in gene activation is much less understood. Forced expression of ncRNAs through *D. melanogaster* PREs causes homeotic phenotypes, suggesting a role in gene activation via the displacement of PcG proteins[60]. Further evidence for an activating role of ncRNAs came from a study of the *bithoraxoid* (*bxd*) PRE and TRE, which encodes a ncRNA that alters *Ultrabithorax* (*Ubx*) gene expression. This study suggested that *bxd* ncRNA transcripts can recruit the methyltransferase ASH1 to *Ubx* in *cis* to induce its expression[61]. However, a conflicting study reported that transcription through the *bxd* PRE and TRE prevents the expression of *Ubx*[38]. The cause for this discrepancy is unclear[62].

Although the above findings suggest that RNA molecules may interact with TrxG components at their site of transcription, more recent reports showed that long ncRNAs (lncRNAs) have an enhancer-like function, acting over long distances to activate gene expression[63]. The precise mechanism by which enhancer-like RNAs mediate gene activation is not known, but it is tempting to speculate that transcription of ncRNAs from enhancers facilitates the contact of the enhancer with its target promoter via chromatin looping. A recent report provided the first evidence that lncRNAs might function to activate transcription by recruiting TrxG complexes to chromatin: *HOXA* transcript at the distal tip (*HOTTIP*), a lncRNA from the 5′ end of *HOXA* locus, was shown to interact with WDR5, targeting WDR5–MLL complexes across *HOXA* to induce H3K4 trimethylation and gene activation. Chromosome looping brings *HOTTIP* RNA in close proximity to its target genes[53] (FIG. 2c). Future work should clarify whether lncRNAs have a general role in recruiting TrxG proteins for gene activation.

## Histone marks and TrxG recruitment

Pre-existing histone modifications may recruit TrxG complexes by providing docking sites for specific 'reader' modules. The third plant homeodomain finger (PHD finger) of MLL binds directly to dimethylated Lys4 on histone H3 (H3K4me2) and H3K4me3 and is necessary for MLL1 recruitment to the *HOX9A* gene locus[64] (FIG. 2d). The NURF complex can also read H3K4me3 via the PHD finger of its largest subunit, NURF301–BPTF (bromodomain and PHD finger-containing transcription factor), thereby linking H3K4me3-mediated gene activation with nucleosome remodelling[65]. The chromodomains of human CHD1, CHD7 and CHD8 interact with methylated H3K4, and SWI/SNF complexes bind to acetylated histones via their bromodomain subunits[66,67]. Furthermore, additional acetylation of H4K16 by the MOF acetyltransferase increases the affinity of BPTF for nucleosomes via the PHD-adjacent bromodomain[68]. However, the precise molecular mechanism of this *trans* histone code remains elusive.

One further example of this histone crosstalk in TrxG-mediated gene activation is the requirement of H2B monoubiquitylation for proper H3K4 methylation and the interplay between the methylation of Arg2 on H3 (H3R2), H3K4 methylation, H3K9 acetylation and H3K14 acetylation. The human PAF complex recruits the H2B ubiquitylation machinery, BRE1–RAD6, which ubiquitylates the H2B tail; this modification enhances H3K4 dimethylation and trimethylation by inducing the catalytic activity of COMPASS[69] (FIG. 3a). Acetylation of H3K9 and H3K14 can also stimulate H3K4me3 (REF. 70) (FIG. 3a), whereas asymmetric H3R2 methylation by protein Arg *N*-methyltransferase 6 (PRMT6) antagonizes H3K4 methylation[71,72] (FIG. 3b). One key player in this crosstalk might be WDR5, which participates in both reading and 'writing' of the H3K4 methylation mark[73]. WDR5 recognizes H3K4 tails independently of their methylation status but is blocked by methylation of H3R2 (REF. 74). Knockdown of *WDR5* results in reduced levels of histone H3K9 and H4K16 acetylation at a



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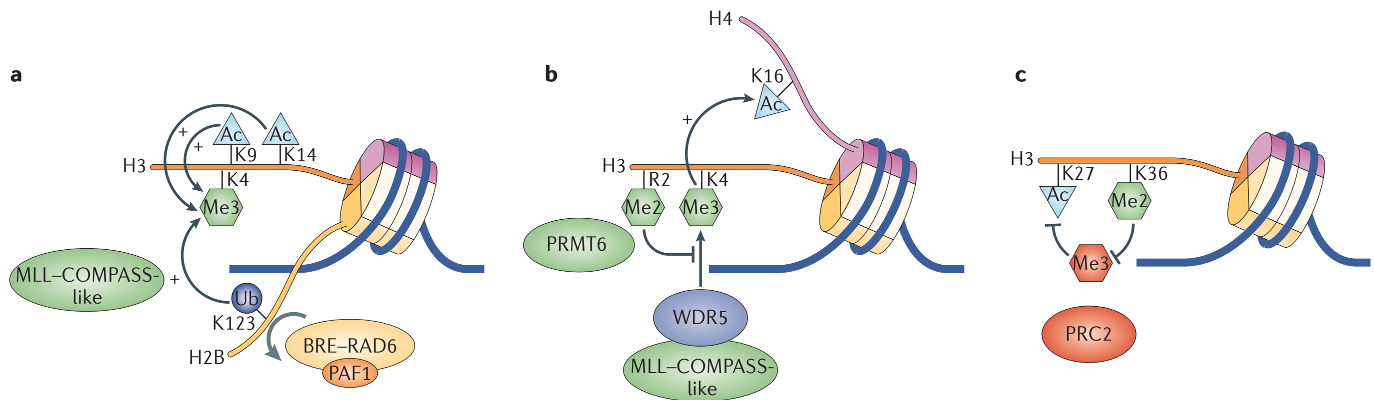


Figure 3 | **Histone crosstalk in TrxG-mediated gene activation. a** | Monoubiquitylation of Lys123 on histone H2B (H2BK123ub) by BRE–RAD6 enhances trimethylation of Lys4 on histone H3 (H3K4me3) by inducing the catalytic activity of COMPASS (complex proteins associated with Set1). Acetylation of Lys9 and Lys14 on histone H3 (H3K9ac and H4K14ac) are also required for maximal H3K4me3 levels. **b** | Asymmetric dimethylation of Arg2 on histone H3 (H3R2me2) by protein Arg *N*-methyltransferase 6 (PRMT6) interferes with binding of WD repeat-containing 5 (WDR5) to tails on histone H3. This results in impaired recruitment of MLL–COMPASS-like complexes, leading to decreased H3K4me3 levels and a subsequent decrease in transcription. **c** | Modification with the H3K27ac mark by Trithorax group (TrxG) complexes containing the histone acetyltransferase cyclic AMP response element-binding protein (CBP) can interfere with trimethylation of the same Lys residue by Polycomb repressive complex 2 (PRC2), thereby blocking Polycomb group (PcG)-mediated gene silencing. In addition, H3K36me2 negatively regulates H3K27me3 levels, helping to keep genes active.

subset of silent genes marked by H3K4me3, suggesting a crosstalk between H3K4 methylation and histone acetylation[75]. However, as WDR5 is also part of complexes that include the HAT MOF, which mediates H4K16 acetylation[26], this crosstalk needs further validation[76].

## TrxG-mediated gene activation

The relative roles of the various TrxG complexes in regulating global gene activation versus specifically counteracting PcG-dependent gene silencing are still ill-defined. In yeast, H3K4me3 occurs after the establishment of the basal transcription machinery and might boost transcription by facilitating elongation and helping to release RNA polymerase II from the promoter. Similarly, the main function of TrxG proteins in higher eukaryotes seems to be to activate transcription downstream of transcriptional initiation. *D. melanogaster* TAC1 has been reported to be essential for the recruitment of the FACT (facilitates chromatin transcription) elongation complex to the *Ubx* gene[38]. TRX and ASH1 bind the coding regions of the active *Ubx* gene[47,77], and their recruitment was suggested to maintain a non-repressive chromatin state in order to prevent PcG gene silencing[78]. Finally, KIS-L acts downstream of Positive transcription elongation factor b (P-TEFb) recruitment to promote early elongation[15]. In addition to transcriptional elongation, ASH2 can regulate transcriptional pausing by affecting the phosphorylation status of RNA polymerase II[79], and H3K4me3 mediated by COMPASS-like complexes can promote transcriptional initiation by directly recruiting the PHD finger of the transcription factor IID (TFIID) subunit TBP-associated factor 3 (TAF3)[80].

Mammalian MLL1 and *D. melanogaster* TRX are proteolytically cleaved by the Thr aspartase Taspase 1 into a SET domain-containing carboxy-terminal domain and an amino-terminal part[81]. Genome-wide mapping studies revealed that the N-terminal fragment of TRX associates with promoters like general transcription cofactors[38,82] and that it also coats the whole coding region of active genes, supporting a role for it in transcriptional elongation[77]. By contrast, the C-terminal fragment of TRX colocalizes strongly with PcG proteins, suggesting a more specific anti-silencing function[62,77].

In addition to H3K4 trimethylation, other histone modifications contribute to TrxG function. The *D. melanogaster* HAT CBP associates with ASH1 and was found in the TAC1 complex, which can acetylate H3K27 in a TRX-dependent manner. H3K27 acetylation was shown to be associated with PcG target genes when they are active[77]. This modification is incompatible with H3K27me3, as these modifications occur on the same amino acid, suggesting that TRX- and ASH1-associated CBP might be a key player in counteracting PcG-dependent gene silencing[37]. Similarly, ASH1-mediated methylation of H3K36 antagonizes PcG-mediated H3K27 methylation[40] (FIG. 3c).

## TrxG-mediated cell cycle regulation

Although TrxG complexes are known to mediate stable epigenetic inheritance, they also regulate gene expression in dynamic processes, such as the cell cycle. Thus, their deregulation may contribute to human leukaemia.

***TrxG-dependent control of cell cycle regulators.*** Leukaemogenic MLL translocations can fuse the common 1,440 N-terminal amino acids of MLL in frame with more than 60 partners. The resulting fusion proteins lose the SET domain. Therefore, they must activate their target genes independently of H3K4 methylation. Recently, it has been proposed that leukaemogenic fusion proteins aberrantly activate MLL targets by constitutively recruiting elongation factors of transcription



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to their promoters[83–85], resulting in their inappropriate expression. Moreover, it has also been demonstrated that mouse acute myeloid leukaemia (AML) tumours generated by expression of an inducible MLL–AF9 fusion can be reverted by conditional MLL–AF9 removal. This addiction of AML tumours to MLL–AF9 expression is predominantly associated with enforcement of a MYB-regulated transcriptional programme that is required for the maintenance of leukaemia[86].

The overexpression of one of two common MLL fusions, MLL–AF4 and MLL–AF9, which are respectively responsible for AML and acute lymphoid leukaemia (ALL), was shown to modify the normal cell cycle in *D. melanogaster*[87] and mammals[88]. In mammals, MLL–AF4 and MLL–AF9 bind the promoter of the *p27* (also known as *KIP1* and *CDKN1B*) CDKI (cyclin-dependent kinase (CDK) inhibitor). However, the outcome of *p27* regulation by MLL–AF4 depends on the cell type. When inducible MLL–AF4 is expressed in an epithelial cell background, MLL–AF4 downregulates the transcription of *p27,* but not of other CDKIs, such as *p21* (also known as *CIP1, CDKN1A* and *WAF1*) or *p16* (also known as *INK4A* and *CDKN2A*)[88]. Conversely, in a lymphoid cell background or in primary bone marrow progenitor cells, MLL–AF4 upregulates *p27* expression[88].

The expression of individual cell cycle regulatory *INK4* genes (*p16*, *p15* (also known as *INK4B* and *CDKN2B*), *p18* (also known as *INK4C* and *CDKN2C*) and *p19* (also known as *INK4D* and *CDKN2D*)) is transcriptionally regulated in a tissue- and developmental stage-specific manner[89,90] that involves several TrxG members. In particular, the menin-containing MLL complex transcriptionally activates the *p27* and *p18* loci in murine embryonic fibroblasts[91,92]. Menin-knockout mice develop multiple endocrine tumours[93–95], correlating with the repression of CDKI in the pancreas, accelerated S phase entry and enhanced cell proliferation in pancreatic islets[95]. Moreover, menin interacts with activator of S phase kinase (ASK; also known as DBF4) and represses ASK-induced cell proliferation[96]. Intriguingly, knocking down MLL1 or retinoblastoma-binding protein 5 (RBBP5; a subunit of the COMPASS complex, see FIG. 1b) in human fibroblasts reduces the expression of a different subset of CDKIs[97,92]. Together with the only partial genome colocalization of menin, MLL1 and RBBP5 (REF. 98), these results open the perspective of context-dependency and differential regulation of CDKIs by different MLL complexes.

*MLL5* is a mammalian TrxG gene that is distantly related to the human augmenter of liver regeneration (ALR) and ASH1-like (ASH1L) proteins and *D. melanogaster* ASH1 (REF. 99). *MLL5* has been recently shown to be a quiescence-induced gene in undifferentiated satellite mouse myoblasts[100], where it both suppresses inappropriate expression of S phase-promoting genes and maintains the expression of fate-determination genes[101]. The proliferation effects of MLL5 may be due to its direct and indirect roles in cyclin A repression[101]. MLL1, MLL2 and SET1 directly associate with E2F to control G1 phase cell cycle genes[102]. Different E2F proteins act as either transcriptional activators or repressors, and MLL1 interacts with E2Fs that are partially different from those of MLL2, suggesting unique, yet redundant, parts played by individual MLLs in cellular division (FIG. 4a). The combinatorial interactions between MLL complexes and E2F proteins may thus contribute to the activation or repression of different E2F target genes in addition to cyclins and CDKIs. Taspase 1-mediated proteolytic cleavage of MLL also regulates cell cycle progression[103]. Taspase 1-null mice are small because of impaired cell proliferation, which is associated with transcriptional downregulation of a subset of cyclins. Finally, the proto-oncogene ASH2 forms a ternary complex with E2F1 and host cell factor 1 (HCF1) on E2F-responsive promoters during the G1–S phase transition, thereby inducing transcriptional activation and cell proliferation[102].

***Regulation of TrxG components by the cell cycle.*** The TrxG–cell cycle connection is not a one-way process. The SWI/SNF chromatin-remodelling complex is itself regulated by the cell cycle, with reduced levels during mitosis and loss of binding to mitotic chromosomes after its dephosphorylation[104,105]. MLL is periodically degraded during the cell cycle (FIG. 4a) by the action of the SCF and APC/C complexes, involving the cell cycle E3 ubiquitin ligases $SCF^{SKP2}$ (SCF bound to S phase kinase-associated protein 2) in S phase and $APC/C^{CDC20}$ (APC/C bound to its co-activator CDC20) during mitosis[12]. Perturbation of this cyclic MLL expression results in cell cycle aberrations, suggesting that the periodicity in MLL expression might be one determinant of the periodicity of cyclins[106]. Remarkably, whereas MLL degradation is initiated in its N terminus, the prevalent MLL fusions exhibit resistance to degradation. This protein stabilization may ultimately result in constant activation of MLL target genes, which could explain the universal consequence of MLL fusions.

Taken together, these data suggest that the differential phenotypes of individual TrxG mutations on cell proliferation may depend on the relative levels of TrxG components, of their cofactors and of the components of the cell cycle machinery in the particular cells, tissues or developmental times under study.

## Senescence and DNA damage checkpoints

Owing to their ability to control CDKI levels, MLL family proteins are an integrative element of the senescence and DNA damage checkpoints. The cellular senescence checkpoint acts as a barrier to cellular transformation[107,108]. During this process, the checkpoint component *p16* is activated by MLL complexes that counteract PcG activity[97]. In young cells, the *p16* locus recruits PcG proteins via the tumour suppressor retinoblastoma protein (RB)[109,110]. In the absence of RB activity in primary human fibroblasts, H3K4me3 levels increase at the *p16* locus, which correlates with transcriptional activation[97], suggesting a balanced action of PcG and TrxG complexes during ageing.

MLL1 could also regulate the *p16* gene after DNA damage and oncogene activation. The ultraviolet-damaged DNA-binding protein DDB1 is able to bind the WD40 proteins RBBP5 and WDR5, and its depletion

**CDKI**
(Cyclin-dependent kinase inhibitor). Members of the CIP and KIP family of CDKIs (p21, p27 and p57) inhibit CDK2- and CDK1-containing complexes, and members of the INK4 family (p15, p16, p18 and p19) inhibit cyclin D-containing complexes. Expression of CDKIs generally causes growth arrest and, when CDKIs are acting as tumour suppressors, may cause cell cycle arrest and apoptosis.

**SCF and APC/C**
(Skp–cullin–F box and anaphase-promoting complex (also known as the cyclosome)). Multiprotein E3 ubiquitin ligase complexes that are involved in the recognition and ubiquitylation of specific cell cycle target proteins for proteasomal degradation.

**E3 ubiquitin ligases**
Enzymes that target specific proteins for degradation by the proteasome by causing the attachment of ubiquitin to Lys residues on their substrates.

**Cellular transformation**
A change undergone by animal cells, caused by escape from control mechanisms (for example, upon infection by a cancer-causing virus). Transformed cells have increased growth potential, alterations in cell surface, karyotypic abnormalities and the ability to invade and metastasize.



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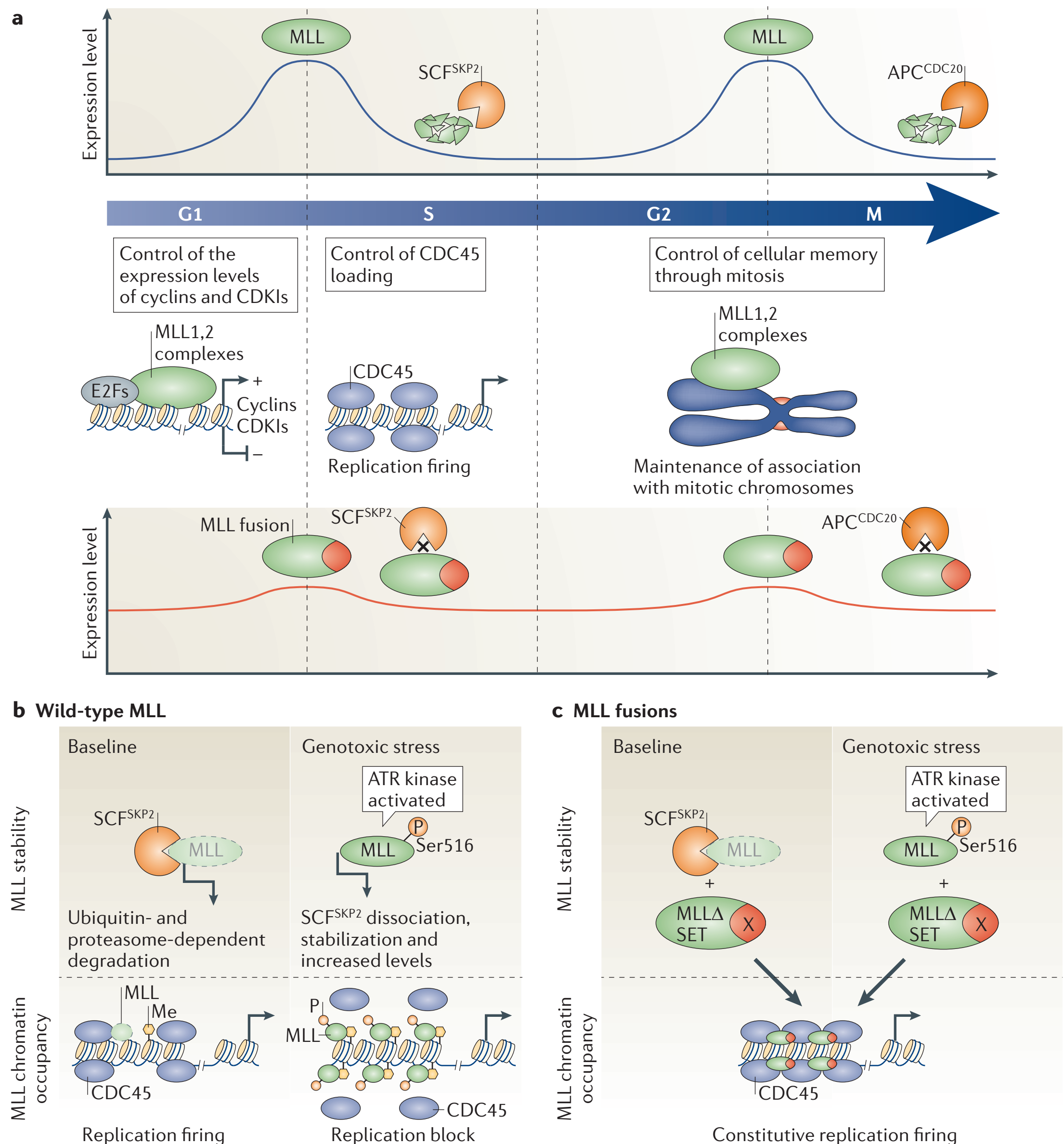


Figure 4 | **Role of MLL proteins in cell cycle control. a** | Under normal conditions, wild-type MLL is unstable and present as peaks of expression at the G1–S and G2–M transitions. The cell cycle E3 ubiquitin ligases $SCF^{SKP2}$ (SCF bound to S phase kinase-associated protein 2) and $APC/C^{CDC20}$ (APC/C bound to its co-activator CDC20) target MLL for degradation during S phase and mitosis, respectively. During G1, MLL1 or MLL2 and SET-domain containing 1 (SET1) directly associate with E2F to control G1 phase cell cycle genes[102]. E2F1, E2F2 and E2F3A activate transcription, whereas E2F3B, E2F4, E2F5 and E2F6 repress it. MLL1 interacts with E2F2, E2F4 and E2F6, and MLL2 associates with E2F2, E2F3, E2F5 and E2F6, suggesting unique, yet redundant, roles for individual MLLs in cellular division. The interactions between MLL complexes and E2F proteins may activate or repress E2F cell cycle target genes in addition to cyclins and cyclin-dependent kinase (CDK) inhibitors (CDKIs). During S phase, MLL degradation results in decreased trimethylation of Lys4 on histone H3 (H3K4me3) on late replication origins, which permits the initiation of DNA replication by the accumulation of CDC45 on DNA. During mitosis, the MLL complex is retained on chromatin, which could facilitate the inheritance of active gene expression during cell division (see BOX 2). In leukaemia, MLL fusions exhibit resistance to degradation and therefore remain at a constant level[12]. **b** | MLL is implicated in the ATR (ataxia-telangiectasia- and RAD3-related)-dependent checkpoint. Under baseline conditions, wild-type MLL protein is degraded during S phase and replication is fired. After genotoxic stress, ATR phosphorylates MLL at Ser516, disrupting the interaction of MLL with $SCF^{SKP2}$ and therefore preventing $SCF^{SKP2}$-mediated MLL degradation. Consequently, MLL accumulates on S phase chromatin at DNA breaks, where it methylates H3K4 and diminishes CDC45 loading to block replication. **c** | MLL dysfunction results in S phase checkpoint defects. In this model, even in the absence of genotoxic stress, levels of MLL fusions might be stabilized owing to the inability of chimeric proteins to bind $SCF^{SKP2}$. Therefore, they could compete with wild-type MLL for loading onto chromatin. MLL fusions that have a SET domain deletion (MLLΔSET) cannot trimethylate H3K4. As a result, CDC45 might load aberrantly at late replication origins. This mechanism could also occur under genotoxic conditions, where MLL fusions would act as a dominant-negative form of wild-type MLL.



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reduces *p16* expression concomitantly with a decrease in H3K4me3 on the *p16* promoter. After transduction of the oncogenic form of HRAS, in which Gly12 is replaced with Val (HRASG12V), into human primary fibroblasts, PcG binding to *p16* is disrupted and *p16* mRNA levels increase[97]. In the same context, deletion of MLL1 or DDB1 reverts the HRASG12V-mediated induction of p16, identifying these proteins as critical components of oncogene-induced *p16* activation[97]. In conclusion, MLL1 and WDR5–RBBP5–DDB1 complexes drive oncogene-induced p16 activation. Furthermore, the SWI/SNF complex was shown to evict PcG complexes and recruit MLL1 at the *p16* gene in malignant rhabdoid tumour (MRT)-derived cells[111], illustrating how multiple TrxG complexes can cooperate to switch genes from repressed to active states.

MLL1 also has a direct role in the control of the S phase checkpoint, which senses DNA damage, inhibits the firing of late replication origins and recruits repair machineries in order to maintain genome integrity[112] (FIG. 4b). After DNA damage, MLL is phosphorylated on Ser516 by the checkpoint kinase ATR (ataxia-telangiectasia- and RAD3-related), reducing S phase-dependent MLL degradation[112]. MLL thus accumulates on chromatin and increases the levels of H3K4me3 on late replication origins, thereby inhibiting chromatin loading of CDC45, an essential component of the pre-replication complex necessary for initiation of DNA replication. MLL mutations impair H3K4 trimethylation and allow CDC45 to load onto damaged chromatin, inducing radioresistant DNA synthesis (RDS). In leukaemias, MLL fusion proteins may act in a dominant-negative manner. For instance, the severity of the RDS partial phenotype of a heterozygote $MLL^{+}/MLL^{ex7(stop)}$–CBP mutant, expressing only the N-terminal part of MLL, is increased by generation of an MLL fusion with CBP ($MLL^{ex7}$–CBP) after conditional excision of the transcriptional stop cassette[113]. Furthermore, the expression of MLL–AF4 or MLL–AF9 fusion protein results in a RDS phenotype despite the cells harbouring two wild-type copies of MLL[113]. The dominant-negative effect of MLL fusion proteins involves their insensitivity to inhibition by ATR-dependent MLL phosphorylation. The chimeric proteins are stabilized even in baseline conditions owing to their diminished interaction with $SCF^{SKP2}$. They thus compete with wild-type MLL for loading onto chromatin and their lack of a catalytic SET domain allows increased CDC45 loading and RDS[112] (FIG. 4c).

A further TrxG contribution to the DNA damage response depends on menin. *Menin*-deficient *D. melanogaster* strains and mouse embryonic fibroblasts are hypersensitive to crosslinking agents and ionizing radiation[114,115] and continue to synthesize DNA after exposure to doses of ionizing radiation that normally causes S phase arrest in wild-type cells[114,116]. Menin is implicated both in the intra-S checkpoint and in the G1–S transition checkpoint. In menin-mutant cells, the upregulation of *p21*, the primary target of activated p53 in the DNA damage checkpoint response, is impaired even though p53 binding is retained. Menin binds and recruits MLL to the *p21* promoter in a DNA damage-dependent manner. Of note, the MLL–menin complex targets *p21* only in the DNA damage checkpoint, whereas in the absence of damage it targets the *p18* and *p27* CDKI genes[91], further highlighting the context-dependency of MLL targeting. Menin also interacts with proteins involved in DNA repair, such as replication protein A, CHK1 and the Fanconi anaemia protein FANCD2, suggesting that, in addition to regulating RDS and the expression of DNA damage-dependent CDKIs, it might play a direct part in DNA repair mechanisms[115,117,118].

## TrxG proteins in signalling to chromatin

Similarly to DNA damage-induced signalling to MLL through ATR-mediated phosphorylation, TrxG components respond to and regulate major signalling pathways linked to developmental and physiological cues.

***TrxG and Notch signalling.*** Research in *D. melanogaster* and mammals suggests that regulation of the Notch signalling pathway engages the antagonistic functions of TrxG and PcG complexes (FIG. 5a). The SWI/SNF complex has been linked to the Notch–Delta transduction pathway[119,120]. In mammals, the interaction of the human SWI/SNF protein BRM with the intracellular domain of the activated form of Notch might promote the transcriptional induction of Notch targets[120]. *D. melanogaster* TRX was also shown to collaborate with Notch in gene activation, although the underlying molecular mechanism is not known[121]; and, intriguingly, one of the leukaemogenic MLL fusion proteins links MLL with the Notch co-activator mastermind-like 2 (MAML2)[122,123].

The histone demethylase UTX, which specifically demethylates H3K27me3, is a tumour suppressor in flies and mammals[124,125]. In *D. melanogaster*, UTX inhibits cellular growth by suppressing the Notch pathway in a RB family-dependent manner[125]. Importantly, mutations in *D. melanogaster Enhancer of zeste* or in *Pc*, respectively affecting a subunit of the PRC2 and the PRC1 complexes, revert the growth defect of *UTX*-mutant cells, suggesting that UTX suppresses tumours through regulation of PcG target genes, possibly including Notch pathway components. Considering that Notch, as well as several upstream and downstream elements of the Notch signalling pathway, has recently been shown to be directly repressed by the PRC1 protein Polyhomeotic[126,127], *D. melanogaster* UTX may affect Notch signalling via the downregulation of a Notch inhibitor. Indeed, a decrease in the mRNA levels of known negative regulators of Notch, such as *numb* and *roughened eye* (*roe*), was observed in *UTX* mutants[125]. Therefore, histone modifiers and chromatin remodellers coordinately orchestrate the transcriptional programme underlying the Notch signal transduction pathway (FIG. 5a). A remarkable fact emerging from these results is that both PcG and TrxG members can either repress or induce Notch signalling, suggesting that a fine regulatory balance ultimately determines the Notch signalling output.

ATR
(Ataxia-telangiectasia- and RAD3-related). A caffeine-sensitive, DNA-activated protein kinase that is involved in DNA damage checkpoints.

Radioresistant DNA synthesis
(RDS). When mutant cells fail to repress the firing of DNA replication origins in the presence of ionizing radiation-induced DNA damage.

Notch signalling
This pathway is a highly conserved intercellular signalling mechanism that is essential not only for cell proliferation but also for numerous cell fate-specification events.



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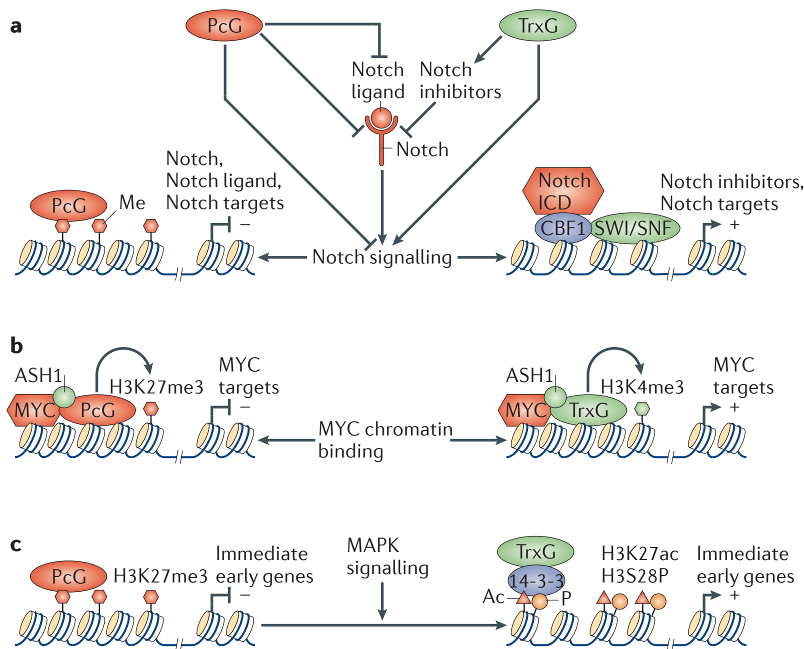


Figure 5 | **Cell signalling and MLL.** Three examples illustrate the subtle interplays existing between the antagonistic functions of the Polycomb group (PcG) and Trithorax group (TrxG) complexes and major signalling pathways. Signalling pathways can act downstream of PcG–TrxG control mechanisms, can help the recruitment of PcG–TrxG complexes to their target genes or can themselves regulate PcG–TrxG activities. **a** | A model for PcG- and TrxG-dependent control of the Notch signalling pathway. Pc repressive complex 1 (PRC1) regulates cell cycle progression via direct repression of the Notch signalling pathway at all levels of its hierarchy. Among the TrxG complexes, the UTX histone-demethylase complex is able to activate inhibitors of the Notch receptor, whereas the SWI/SNF (switch/sucrose nonfermentable) chromatin-remodelling complex associates and collaborates with the activated Notch intracellular domain (ICD) to activate Notch targets. The Notch ICD is recruited to chromatin via CBF1. **b** | MYC-dependent targeting of PcG and TrxG complexes controls MYC-induced growth. On the one hand, MYC binds to components of the PRC1 complex and shares common targets, among which is MYC itself. On the other hand, MYC associates with TrxG complexes, like Absent small and homeotic discs 1 (ASH1) and ASH2-containing complexes, and trimethylates Lys4 on histone H3 (H3K4me3). MYC target gene promoters also reveal a strong dependency on the H3K4me3 status for E box-dependent MYC binding. Interestingly, the TrxG protein ASH1 can at the same time assist the PcG-dependent repression of a subset of MYC targets and participate in the TrxG-dependent activation of another subgroup of MYC targets. **c** | Mitogen-activated protein kinase (MAPK) signalling acts upstream of PcG and TrxG to coordinate their antagonistic functions on immediate early genes. MAPK signalling cascades induce a nucleosomal response by a chromatin-remodelling mechanism, which is dependent on phosphorylation of Ser28 on histone H3 (H3S28P). This phosphorylation triggers eviction of PcG complexes from chromatin and, concomitantly, transcriptional activation of immediate early genes. The H3S28 phosphorylation could cause an epigenetic switch from the repressed H3K27me3 mark to the active mark, acetylation of Lys27 on histone H3 (H3K27ac).

Extracellular signal-regulated kinase
(ERK). A protein involved in a mitogen-activated protein kinase signal transduction pathway that functions in cellular proliferation, differentiation and survival. Its inappropriate activation is a common occurrence in the human cancers.

***TrxG and MYC regulation.*** Another case of interplay between TrxG and PcG proteins concerns the regulation of MYC function (for a review, see REF. 128). In *D. melanogaster*, Little imaginal discs (LID) acts as a co-activator of MYC[129]. LID is an atypical TrxG component, as it carries an H3K4 demethylase activity. MYC binds LID and inhibits its demethylase activity, and LID association is required for MYC-dependent transcriptional activation. This suggests that the H3K4 demethylase is not responsible for the TrxG function of LID. The LID–MYC relationship is most likely evolutionarily conserved, because the mammalian LID orthologues, jumonji and ARID domain-containing 1A (JARID1A; also known as KDM5A and RBP2) and JARID1B (also known as KDM5B and PLU1), also bind MYC. MYC also interacts with other TrxG complexes, such as the SNF5 component of the SWI/SNF complex[130] and ASH2 (REF. 129). Intriguingly, however, MYC might also interact with PcG complexes[131]. In *D. melanogaster*, a set of MYC target genes is repressed by Pc in the presence of the TrxG protein ASH1, whereas a second set of MYC targets is co-activated by ASH1 (REF. 132). MYC might therefore use PcG and TrxG proteins to drive its target genes into a repressed or an active mode, and it is conceivable that the modulation of MYC interactions with PcG or TrxG members may enable MYC to control genes involved in growth and proliferation (FIG. 5b).

***TrxG and MAPK signalling.*** Although much about the molecular roles of TrxG proteins in Notch signalling and MYC function remains to be learned, recent work has paved the way for the elucidation of TrxG and PcG function in regulating cellular commitment to the muscle lineage. The p38 mitogen-activated protein kinase (MAPK) signalling pathway activates muscle-specific genes during the commitment of myoblasts into multinucleated myotubes. In growing myoblasts, muscle-specific genes are epigenetically marked for repression by H3K27me3 (REF. 133). During differentiation, the myocyte-specific enhancer factor 2D (MEF2D) isoform of the MEF2 muscle-specific transcription factor is phosphorylated by the MAPK p38α (also known as MAPK14), stimulating its interaction with the MLL2 complex[134]. This promotes targeting of the MLL2 complex to muscle-specific genes, such as myogenin (*MYOG*) and creatine kinase M-type (*CKM*), leading to their H3K4 trimethylation and activation[134].

In addition to stimulating H3K4 trimethylation, the extracellular signal-regulated kinase (ERK) and p38 MAPK pathways are also involved in recruiting SWI/SNF chromatin-remodelling complexes to the *MYOG* and *CKM* promoters[135]. PcG binding is also affected by this MAPK-dependent chromatin remodelling. Phosphorylation of histone H3 Ser28 (H3S28P) by nuclear mitogen- and stress-activated kinase 1 (MSK1) and MSK2, which transduce MAPK signalling, results in the eviction of PcG complexes from chromatin[136,137]. Subsequent to this MSK1-mediated nucleosomal response, BRG1 can be recruited to the H3S28P mark via its association with the 14-3-3 phosphobinding protein[138], thus enabling the binding of transcription factors. Together, these data suggest a scenario in which MAPK induction can simultaneously remove PcG components and induce the recruitment of H3K4-methylating and chromatin-remodelling complexes to induce gene expression (FIG. 5c).

TrxG components regulate, and are regulated by, the other main signalling pathways in mammals, flies and *C. elegans*, including RAS signalling[32], steroid hormone-mediated signalling (reviewed in REF. 139) and signalling by the WNT–β-catenin pathway[140–142]. Remarkably, signalling pathways can act upstream as well as downstream



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Nucleosomal response
The rapid phosphorylation of histone H3 that occurs concomitantly with the induction of immediate early genes, which is mediated through alternative mitogen-activated protein kinase cascades.

WNT–β-catenin pathway
A signalling pathway involving widely conserved secreted signalling molecules of the Wingless family, which regulate many processes during animal development.

JAK–STAT
(Janus kinase–signal transducer and activator of transcription). A rapid signal transduction pathway used by a range of cytokines and growth factors. Binding of a cytokine or growth factor to its receptor activates cytoplasmic JAK, which then phosphorylates STAT and triggers its translocation into the nucleus, where it induces the transcription of specific genes.

Neural progenitor cells
(NPCs). A stem cell type found in adult neural tissue that can give rise to neuron and supporting cells (glia). During development, NPCs produce the enormous diversity of neurons and glia in the developing central nervous system, and they have been also shown to engage in the replacement of dying neurons.

Long-term HSCs
(LT-HSCs). Haematopoietic stem cells that have long-term regeneration capacities and can restore the haematopoietic system of an irradiated mouse over months.

Short-term HSCs
(ST-HSCs). Haematopoietic stem cells that, under normal circumstances, cannot renew themselves over a long term. They are also referred to as progenitor or precursor cells, as they are relatively immature cells that are precursors to a fully differentiated cell of the same tissue type.

of TrxG complexes. For instance, *D. melanogaster* NURF is a negative regulator of the JAK–STAT (Janus kinase–signal transducer and activator of transcription) signal transduction pathway[143,144] and, reciprocally, signalling mediated by inositol polyphosphate second messengers regulates *D. melanogaster* NURF and yeast SWI/SNF[145,146]. The regulatory position of TrxG and PcG components at the crossroad between cell proliferation and differentiation may actually require global control of their activities and targeting properties by signalling pathways to allow the appropriate functional switches to be correctly specified.

## TrxG proteins and stem cells

TrxG proteins and their repressive PcG counterparts have important roles in gene regulation during the maintenance and differentiation of ES cells, which can generate all different cell types during development[147]. ES cell pluripotency and self-renewal are maintained by a core regulatory circuitry, which depends on a limited number of transcription factors, including OCT4 (also known as POU5F1), SOX2, NANOG, oestrogen-related receptor-β (ESRRB), T box 3 (TBX3) and transcription factor 3 (TCF3)[148–151]. These factors work by either repressing lineage-specific genes or activating genes that maintain pluripotency. To do so, they cooperate with chromatin regulatory proteins, including TrxG and PcG components, to reprogramme cell states.

***TrxG and bivalent chromatin domains in ES cells.*** A striking feature of ES cells is that PcG-repressed genes also contain active histone marks, such as H3K4me3, giving rise to so-called bivalent chromatin domains (reviewed in REF. 152). These can then be resolved during lineage commitment and either lose the H3K4me3 mark to become stably silenced or lose the H3K27me3 mark for transcriptional activation[153,154]. MLL proteins have an important role in stem cell functions[8,155–159]. However, as mammals have at least six H3K4 methyltransferases (FIG. 1b), the study of their individual roles in stem cell maintenance and differentiation is a challenging task. For instance, MLL2-depleted ES cells showed no obvious defect in their self-renewal capabilities, although they had an enhanced rate of apoptosis and growth impairment in the undifferentiated state and during the first days of differentiation[25,155]. However, the depletion of essential and non-redundant MLL-complex components has been very informative in this respect[8,156]. In particular, WDR5 was shown to be required for ES cell self-renewal[8] by physically interacting and strongly colocalizing with OCT4 in the genome, including at the promoters of self-renewal genes, where it is necessary to maintain high levels of H3K4me3 and transcriptional activation[7].

***TrxG proteins and adult stem cells.*** After embryonic development, several types of adult stem cells (such as haematopoietic stem cells (HSCs), germline stem cells (GSCs) and neuronal progenitor cells (NPCs)) proliferate to replenish dying cells and regenerate damaged tissues. Using ES cell-derived *in vitro* culture systems, it is possible to trigger neuronal differentiation and therefore investigate the neuronal differentiation process[160]. This showed that the promoters of neural genes are bivalent and repressed in ES cells. During differentiation into NPCs, they lose the H3K27me3 modifications and retain the H3K4me3 marks, resulting in increased expression[161]. The resolution of bivalency is achieved by active H3K27me3-specific demethylation, downregulation of the methyltransferase Enhancer of zeste homologue 2 (EZH2) and dynamic switches in Pc targets[162–166]. In addition, RBBP5 and DPY30, two components of COMPASS-like complexes, are necessary for differentiation of ES cells into NPCs. DPY30- or RBBP5-knockdown in ES cells prevents the differentiation of ES cells into neurons. Depleted cells show a strong reduction in H3K4me3 levels and a reduction in the transcriptional upregulation of the vast majority of genes normally induced in NPCs[156]. These results confirm that core subunits of MLL complexes have an important role in modulating the gene expression programme during the ES cell fate transitions to neurons.

The role of TrxG components extends to other stem cells and, in particular, much has been learned from the study of the haematopoietic system. Most of the promoters of genes involved in the regulation of haematopoietic cell maturation are bivalent and, upon normal differentiation, only a few promoters retain bivalency, whereas on average 15% lose H3K27me3, 50% lose H3K4me3 and the rest lose both marks[167,168]. The massive loss of H3K4me3 suggests that TrxG complexes may have a role in the maintenance of HSCs and their differentiation. Indeed, *Mll1*-knockout mice die at early embryonic stages, and their fetal livers show reduced numbers of long-term HSCs (LT-HSCs), short-term HSCs (ST-HSCs) and quiescent HSCs. Moreover, HSCs of adult mice depleted of MLL1 in the haematopoietic system show proliferation and self-renewal defects, as well as multi-lineage impairment in haematopoiesis[169,170]. Mutations in *Mll2* are also embryonic lethal in mice, but adult mice lacking MLL2 appear normal and do not show any defects in blood profiles, although males and females are sterile[155,171]. Sterility is also observed in flies when temperature-sensitive *Trx*-mutant flies grown at permissive temperature are shifted to non-permissive temperature as adults. In this case, TRX is required during spermatogenesis to maintain H3K4me3 and transcription activation at the promoters of genes coding for tTAFs, testis-specific transcription factors that are required for sperm differentiation[172]. *MLL2*-mutant mice are also sterile owing to a block of spermatogenesis[155], and oocyte death in females is associated with loss of H3K4me3 (REF. 171); therefore, this mark may be generally required for germ cell development. Indeed, H3K4me3 was detected by immunostaining in all germ nuclei in the mitotic and meiotic regions and was strongly reduced in the germ line of mutant animals[173,174].

***TrxG-dependent nucleosome remodelling in stem cells.*** Inactivation of BRG1 in mice results in embryonic lethality and a failure to form pluripotent cells[175,176]. Inactivation of other SWI/SNF complex subunits,



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including BRG1-associated factor 47 (BAF47), BAF57, BAF60, BAF155, BAF180 and BAF250A, also leads to embryonic lethality, whereas inactivation of BRM does not affect viability[175–180]. Intriguingly, ES cells have a unique subunit composition of SWI/SNF complexes, the esBAF complex, which was reported to interact with proteins expressed in the pluripotent state[181]. esBAF BRG1 has been shown to interact with OCT4, suggesting that esBAF might collaborate with master regulators, such as OCT4 and SOX2, to regulate the ES cell transcriptional circuitry[181]. Genome-wide mapping of BRG1 showed 6,000 binding sites. Most of them are highly correlated with H3K4me, H3K27 acetylation and H3K27me3 (REF. 182), and some of them physically overlap with OCT4, SOX2 and NANOG occupancy[181]. Transcriptome analysis upon BRG1 depletion in ES cells showed that BRG1 has a mainly repressive function, in agreement with recent findings in which BRG1 has been mapped to promoter distal regions that are enriched for H3K27me3, a modification that is associated with PcG-mediated silencing[182].

NURF components have also been shown to play a part in ES cell differentiation. Knockout mice for the BPTF component of the NURF complex die early in embryogenesis. Genetic and molecular analyses in ES cells showed that BPTF is not required for cell viability but is essential for endoderm differentiation. Transcriptome analyses of wild-type and *Bptf*-knockout ES cells grown under pluripotency versus differentiation conditions suggest that BPTF may have both repressive and activating functions, even though it is unclear whether these effects are direct[183]. RNA interference-mediated downregulation of CHD1 leads to loss of primitive endoderm and abnormally high levels of neural differentiation. Furthermore, genome-wide mapping of CHD1 showed strong correlation with binding sites of RNA polymerase II and H3K4me3, whereas bivalent domains were largely devoid of CHD1. These data indicate that CHD1 is mostly linked to gene activation required for the establishment of ES cell pluripotency and differentiation[43].

## Conclusions

The role of TrxG components in biology is pervasive. However, different TrxG components have specific and sometimes opposing roles. The main tasks for future research will be to better understand how TrxG complexes are recruited to the whole set of their target genes and to clearly discriminate which TrxG proteins and complexes perform which functions in which cell types and developmental stages. For instance, one essential point will be to separate the general transcription co-activator function of TrxGs from the more specific function in opposing PcG-mediated gene silencing. For this, a systematic genetic study of individual and double mutants will be required, as well as quantitative genome-wide data analyses of the effects of these mutations. Moreover, the signals that induce the specific removal of epigenetic marks during differentiation remain to be identified, as do the mechanisms by which these signals can simultaneously induce repression at certain genes while activating others.

It is still unclear whether the action of TrxG complexes is continuously required during development, or whether there are stages at which cell fate choices may become irreversible and thus no longer require TrxG proteins for their maintenance. The ongoing intense research in this field is likely to lead to a much deeper understanding of these issues in the coming years. This will not only increase our general understanding of epigenetic regulation in eukaryotic organisms but also help refine stem cell and induced pluripotent cell technologies, as well as provide new tools for diagnostics and therapeutic targets for haematopoietic malignancies.

Finally, as TrxG proteins have pleiotropic gene control functions, they are emerging as new players in novel physiological phenomena. For instance, tissue regeneration, longevity and the environmental stress response have been shown to involve the function of TrxG members[13,184,185]. As research progresses, insight into the molecular mechanisms at the base of these functions, and new links to unexpected phenomena, are likely to open avenues in the TrxG and epigenetics field.


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**Acknowledgements**
We apologize to those whose recent publications we were unable to cite owing to space limitations. N.I. was supported by a long-term European Molecular Biology Organization fellowship and by a fellowship from the Human Frontier Science Program Organization. The research of C.G. was supported by grants from the European Research Council (ERC-2008-AdG no. 232947), the Centre national de la recherche scientifique, the European Network of Excellence EpiGeneSys, the Agence Nationale de la Recherche and the Association pour la Recherche sur le Cancer.

**Competing interests statement**
The authors declare no competing financial interests.

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