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The *Arabidopsis* LHP1 protein colocalizes with histone H3 Lys27 trimethylation

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Polycomb proteins are required for maintenance of silent chromatin states via histone H3 Lys27 trimethylation (H3K27me3) in animals, but homologs are not found in plant genomes. Using a DamID-chip method, we found that the *Arabidopsis thaliana* chromodomain-containing protein LHP1 colocalizes with H3K27me3 genome-wide. The LHP1 chromodomain also binds H3K27me3 with high affinity, suggesting that LHP1 has functions similar to those of Polycomb.

Post-translational histone modifications are important in regulating eukaryotic gene expression. Lys9 (H3K9me3) and Lys27 (H3K27me3) trimethylation on the N-terminal tail of histone H3 regulate chromatin structure by promoting interactions with heterochromatin protein-1 (HP1) and the Polycomb (Pc) protein, respectively[1,2]. There are several interesting parallels between HP1 and Pc. First, both bind methyllysine through a conserved N-terminal chromodomain. Second, both interact with the histone methyltransferases responsible for the histone methyl groups they bind; these interactions are essential for maintenance and spreading of H3K9me3 and H3K27me3 modifications. Third, both are required to maintain their target regions in a repressive chromatin state by either recruiting additional factors involved in heterochromatin formation (such as histone deacetylases) or mediating oligomerization[1–3]. Despite these similarities, HP1 and Pc have distinct biological functions, regulating different components of the genome[4].

Plants and animals share the enzymatic components of the H3K9 and H3K27 methylation pathways (the SUVH3–9 and E(Z) protein families, respectively). However, no plant Pc homolog has been described, and sequence homology suggests that *Arabidopsis LIKE HETEROCHROMATIN PROTEIN-1* (*LHP1*, also called *TERMINAL FLOWER-2, TFL2* or *TU8*) encodes an HP1 homolog[5,6]. Based on animal and fungal HP1 homolog function, and observations that LHP1 can bind H3K9-methylated peptides *in vitro*, it has been proposed that LHP1 might be involved in heterochromatic gene silencing in plants[7]. However, although DNA methylation and H3K9me2 (associated with silent genes in plants) are highly enriched in pericentromeric heterochromatin, immunofluorescence studies of LHP1 have revealed predominantly euchromatic signals[8–10]. Further, *lhp1* null mutants show no effect on DNA methylation or transposon silencing[9,11,12], but instead display abnormalities that suggest a role for LHP1 in development. In addition, genes misregulated in *lhp1* mutants are mostly distributed in euchromatin[8,9].

LHP1 localizes to the flowering-repressor gene *FLOWERING LOCUS C* (*FLC*) and is required to maintain the epigenetic silencing of *FLC* during prolonged cold exposure[10,13]. *FLC* is regulated in part by VERNALIZATION-2 (VRN2, a homolog of the Polycomb-group protein Su(z)12)[14], and *FLC* chromatin is associated with H3K27 methylation[10,13]. Four additional LHP1 target genes were also found to be H3K27me3 associated in a genome-wide profile of this histone modification using tiling microarrays[15,16].

To study the function of LHP1, we combined the DNA adenine methyltransferase identification (DamID) method with high-density whole-genome tiling microarrays (DamID-chip) to identify LHP1 target regions in the *Arabidopsis* genome. An *Escherichia coli* Dam and LHP1 fusion was introduced into *Arabidopsis*, so that LHP1 binding to specific chromosomal locations led to preferential adenine methylation at nearby GATC sites[15]. The Dam-LHP1 fusion protein is most probably functionally wild type, as transgenic plants expressing Dam-LHP1 seem normal and its localization to specific loci (such as *FLC*; see **Fig. 1**) is consistent with previous studies[10,15]. Adenine methylation was assayed using a methylation-specific PCR protocol and detected by hybridizing the resulting DNA fragments to tiling microarrays (**Supplementary Fig. 1** and **Supplementary Methods** online)[4,17]. DamID-chip results were validated using a complementary method[15], which yielded highly consistent results (**Supplementary Fig. 2** and **Supplementary Table 1** online).

We identified 2,354 LHP1 regions in the *Arabidopsis* genome, including all known LHP1 bound loci (such as *FLC*; see **Fig. 1**). Genome-wide LHP1 localization data can be viewed at http://epigenomics.mcdb.ucla.edu/LHP1/. Consistent with immunofluorescence studies, LHP1 was frequently found in gene-rich euchromatin and was depleted in the repeat-rich pericentromeric heterochromatin (**Fig. 1b**). Compared with control regions (see **Supplementary Methods** for definition), LHP1 regions were not preferentially associated with heterochromatic epigenetic marks such as DNA methylation or short interfering RNAs (siRNAs) (**Fig. 1c**). LHP1 target genes were also over-represented >2.5 fold ($P < 10^{-5}$; see **Supplementary Methods**) among genes upregulated in the *lhp1* mutant[9]. Together, these results suggest that LHP1 localizes to euchromatin and functions mainly in the suppression of euchromatic genes.

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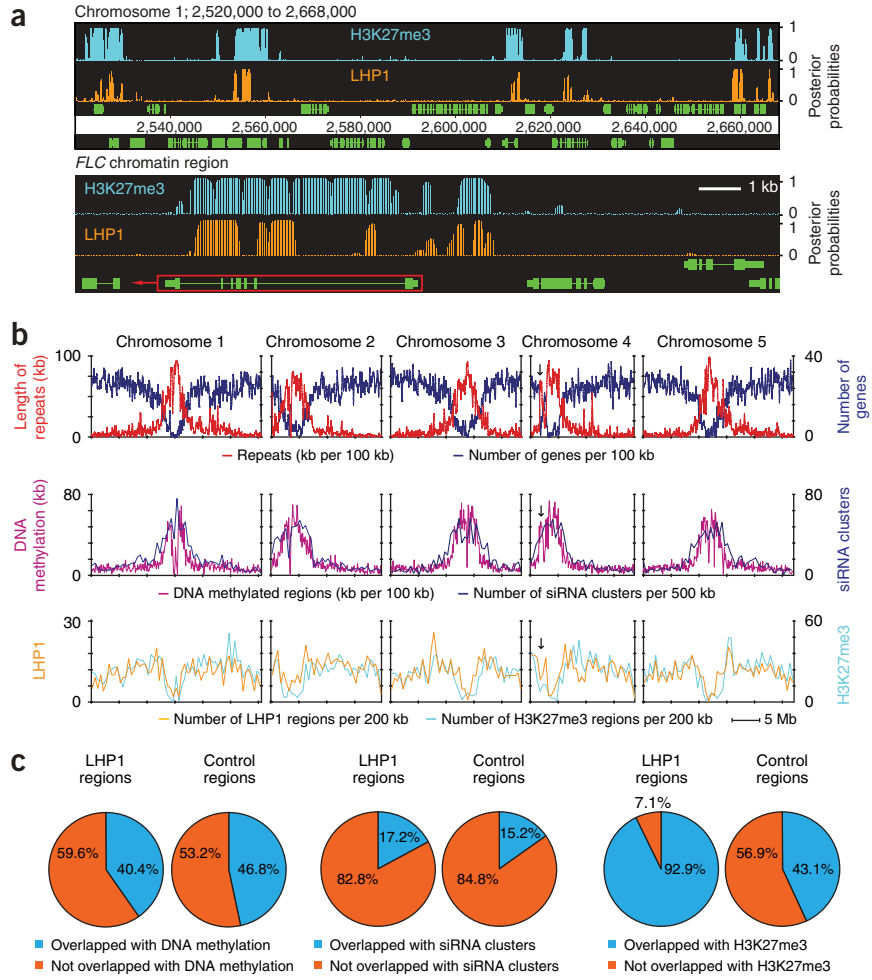

Figure 1 Genome-wide *Arabidopsis* LHP1-binding sites. (**a**) LHP1 (orange) and H3K27me3 (light blue) colocalization in a chromosome 1 region (top) and the *FLC* locus (At5g10140, bottom). Green boxes and lines, gene exons and introns, respectively. Red box, the *FLC* gene; red arrow, direction of transcription. (**b**) Chromosomal distribution of LHP1. Top, enrichment of repetitive sequences in heterochromatin (red) and genes in euchromatin (blue). Middle, heterochromatic distribution of DNA methylation (pink) and siRNAs (blue). Bottom, euchromatic distribution of LHP1-binding sites (orange) and H3K27me3 (light blue). Arrows, chromosome 4 heterochromatic knob. kb, kilobases; Mb, megabases. (**c**) Fractions of LHP1-binding sites that overlapped with given genomic regions.

Notably, the chromosomal distribution of LHP1 highly resembles that of H3K27me3 (**Fig. 1a**,**b**). Of the 2,354 LHP1 regions, 2,186 (~92.9%) were associated with H3K27me3 (**Fig. 1c**). Similarly, of the 2,632 H3K27me3 regions that were longer than 1 kilobase, 2,346 (~89.1%) colocalized with LHP1. In addition, similar to the gene-level distribution of H3K27me3, LHP1 regions were also enriched in the transcribed regions of genes (**Supplementary Fig. 3** online). Such a strong correlation suggests that LHP1 binds chromosomal regions that are associated with H3K27me3 *in vivo*.

To test if the LHP1 chromodomain, which is highly similar those of both the HP1 and Pc families (**Supplementary Fig. 4** online), binds to H3K27me3, we measured the binding affinity of the LHP1 chromodomain for methylated and unmodified peptides. The LHP1 chromodomain had similarly high binding affinities for H3K27me3, H3K9me3, H3K9me2 and H3K9me3K27me3, an approximately ten-fold lower affinity for H3K9me1 and an even lower affinity for unmodified H3 (**Fig. 2**). The high affinity of the LHP1 chromodomain for H3K27me3 is consistent with its colocalization *in vivo* with this epigenetic mark, whereas the significance of its H3K9me3 affinity is unclear, as plants contain very little H3K9me3 according to mass spectrometry[18]. Furthermore, a recent profiling study using chromatin immunoprecipitation coupled with microarray analysis found no correlation of H3K9me3 sites and LHP1-binding sites[19]. Notably, while the LHP1 chromodomain binds H3K9me2 and H3K27me3 with similar affinities, LHP1 tends not to localize with H3K9me2 *in vivo*, as shown by immunofluorescence staining. Similar results were recently described in mice, where several Pc homologs were found to be capable of binding both H3K9me2 and H3K27me3 (ref. 20). Thus, it seems that the binding of the LHP1 chromodomain to methylated histone H3 may facilitate its localization, but other regions in LHP1 or additional factors are involved in the LHP1 targeting. Indeed, previous studies have shown that overexpression of LHP1 results in its ectopic accumulation in H3K9me2 rich heterochromatin.

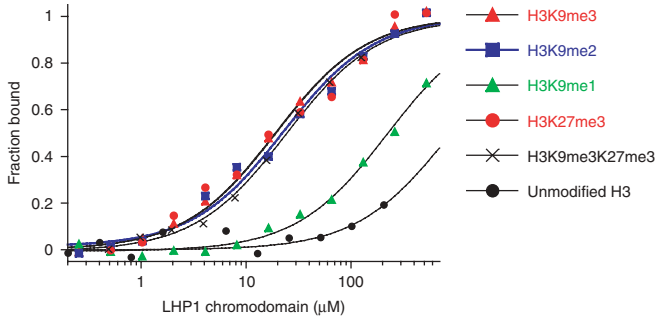

Figure 2 Fluorescence polarization binding assays show that the LHP1 chromodomain binds equally well to H3K27me3 (K_d = 19 ± 2 μM), H3K9me3 (K_d = 19 ± 1 μM), H3K9me2 (K_d = 22 ± 2 μM) and H3K9me3K27me3 (K_d = 25 ± 0.9 μM), but ten-fold less to H3K9me1 (K_d = 227 ± 11 μM) and very poorly to unmodified H3 tail (K_d > 500 μM).

In summary, the results presented here suggest that LHP1 binds genomic regions associated with H3K27me3, probably facilitated in part by the direct interaction between its chromodomain and H3K27me3. This is consistent with a recent study that compared H3K27me3 and LHP1-binding sites on *Arabidopsis* chromosome 4 at a 1-kilobase resolution[19]. In this respect, LHP1 is functionally similar to Pc, a subunit of Polycomb repressive complex-1 (PRC1) previously thought to be missing in plants. Thus, LHP1 may represent the first equivalent of a PRC1 component in plants, and its functional characterization should allow identification of additional plant

PRC1 components not yet found by sequence homology. Finally, the absence of additional proteins belonging to the HP1 or Pc families in plants raises the possibility that distinct mechanisms may be responsible for H3K9 methylation–mediated heterochromatic gene silencing in plants and animals.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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AUTHOR CONTRIBUTIONS

S.K., V.G. and S.E.J. designed the experiments; X.Z., S.G. and B.J.B. performed the experiments and analyzed the data; X.Z. wrote the paper.

COMPETING INTERESTS STATEMENT

The authors declare no competing financial interests.

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