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RESEARCH HIGHLIGHT

ADCP1: a novel plant H3K9me2 reader

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H3K9 methylation is an epigenetic mark associated with heterochromatin. Zhao and colleagues characterize a novel H3K9me reader protein, ADCP1, that plays a role in heterochromatin maintenance and shows functional similarities to animal HP1 proteins.

Eukaryotic genomes are broadly compartmentalized into non-overlapping transcriptionally permissive 'euchromatic' and repressed 'heterochromatic' regions. These regions are decorated by a suite of distinct epigenetic marks, which help to reinforce and demarcate their transcriptional state. In *Arabidopsis*, the majority of heterochromatin is confined to the central portion of each chromosome near the centromere, and this spatial separation from euchromatin makes it an attractive model to study epigenetic states. Di-methylation of lysine 9 of histone H3 (H3K9me2) is a key epigenetic mark associated with heterochromatin in plants, functionally analogous to H3K9me3 in mammals.[1]

A large body of work has described epigenetic feedback loops in *Arabidopsis*, which act to maintain H3K9me2, together with DNA methylation.[1] For instance, three partially redundant proteins, KRYPTONITE (KYP, also known as SUVH4), SUVH5 and SUVH6 work together with CHROMOMETHYLASE3 (CMT3) to form a self-reinforcing feedback loop for coincident maintenance of CHG methylation and H3K9me2. The SRA domain of KYP binds to methylated DNA, and establishes H3K9me2 via its C-terminal SET histone methyltransferase domain. Reciprocally, the N-terminal BAH and chromo domains of CMT3 bind to H3K9me2, and the C-terminal DNA methyltransferase domain establishes CHG methylation. KYP/SUHV5/SUVH6 also operate in a similar feedback loop with CMT2 to maintain CHH methylation. Because these proteins contain both heterochromatic 'reader' and 'writer' domains, they form straightforward epigenetic feedback loops.

Much less is known about factors that act downstream of H3K9me2 to enforce transcriptional repression. Zhao and colleagues describe a novel protein, ADCP1, which by its domain structure, appears to have a strict H3K9me2 'reader' function.[2] However, the authors show that ADCP1 also clearly contributes to H3K9me2 and DNA methylation maintenance, although it is currently unclear whether these effects are direct or indirect. Importantly, the *adcp1* mutant also shows derepression of many transposons marked with H3K9me2, and shows a partial decondensation of the chromocenters (dense nuclear bodies containing the pericentromeric heterochromatin). Interestingly, a recent paper by Zhang and colleagues also identified the same protein from a different proteomics screen (calling it 'AGDP1'), and reported largely similar results.[3]

ADCP1 was initially identified from a previously published lab-on-chip histone reader screen by the same research group.[4] ADCP1 encodes three tandem Agenet domains, which are homologues of Tudor domains. The authors show that each tandem Agenet domain binds H2K9me2. They solve the crystal structure in complex with an H3K9me2 peptide, finding that the tandem domains adopt a 'face-to-face' orientation, and discriminate H3K9me2 via an aromatic cage using a similar mechanism described for other K9me reader proteins. The authors also examine the distribution of ADCP1 in vivo by ChIP-seq, finding a near perfect colocalization with H3K9me2 genome-wide.

The mammalian gene silencing protein HP1, which also binds to H3K9 methylation, was recently shown to form phase-separated droplets in vivo.[5,6] These membrane-less compartments are thought to facilitate heterochromatin function and maintenance, in part by helping to isolate these regions from the surrounding nuclear environment. Zhao and colleagues showed that ADCP1 can also assemble into phase-separated liquid droplets in vitro, a property which was stimulated by the addition of H3K9me3-containing synthetic nucleosomes. Interestingly, both binding to H3K9me2/3, and droplet formation in vitro, was reversed by phosphorylation of serine 10 (S10p). This indicates the potential for a 'phospho-switch' mechanism,[7] whereby heterochromatin could be reversibly dissolved by the action of kinases. As S10p is known to dislodge HP1 from H3K9me3 during mitosis,[8] one could imagine a scenario whereby S10p transiently releases chromatin compaction to facilitate heterochromatin replication during cell division. Another tantalizing possibility is that S10p may allow for the transient release of gene silencing in response to cell signaling, without compromising epigenetic memory.[8] The phosphorylation state of HP1 itself also affects its ability to cause phase separation,[5] and thus it would be interesting to determine whether ADCP1 was also regulated by such a mechanism. However, as a first step it will be important to determine whether ADCP1 forms phase-separated droplets in vivo, and if so, what are the constituents within.

Interestingly, the *adcp1* mutant showed significant losses of both H3K9me2 and non-CG methylation. As ADCP1 appears to only encode H3K9me2 'reader' domains, it is unclear how the *adcp1* mutant leads to a loss of H3K9me2 itself. One possibility is that ADCP1 participates in the feedback loop between SUVH4/5/6 and CMT2/3 to maintain H3K9me2 and non-CG methylation (Fig. 1). However, the histone methyltransferase *suvh4/5/6* triple mutant or the non-CG DNA methyltransferase *drm1/2/cmt2/3* quadruple mutant each completely eliminates H3K9me2 genome-wide,[9] indicating that ADCP1 is not redundant with these factors or sufficient to establish H3K9me2 on its own. It is also worth noting that ADCP1 was not isolated from saturating mutant screens designed to identify factors involved in heterochromatin-

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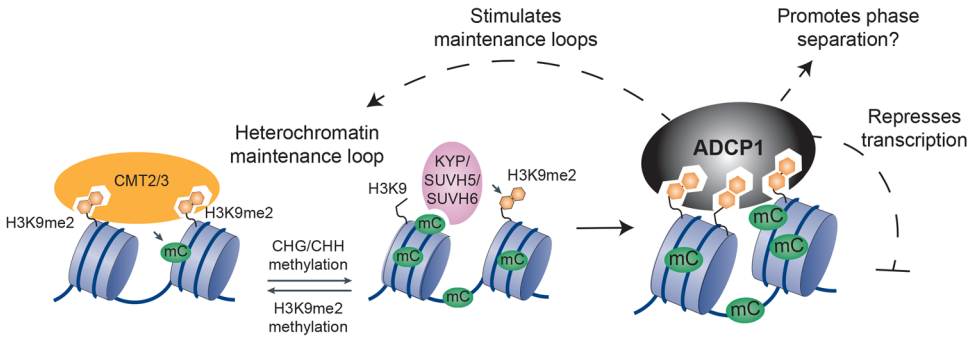

Fig. 1 Model for how ADCP1 contributes to heterochromatin maintenance. The core epigenetic feedback loops are depicted between CMT2 and CMT3 that bind H3K9me2 and methylate DNA (mC), and KYP, SUVH5, and SUVH6 that bind mC and di-methylate H3K9. ADCP1 binds H3K9me2 via three tandem Agenet domains and may promote the maintenance of heterochromatin by three non-mutually exclusive mechanisms. This figure is modified from Fig 2a of a previous study [1]

mediated gene silencing, in which KYP/CMT3 alleles were isolated 30 times.[10,11] Furthermore, Zhang and colleagues performed IP-MS on ADCP1, but did not pull down SUVH4/5/6 or CMT2/3, arguing against direct recruitment.

A second possibility is that H3K9me2 loss is an indirect consequence of transcriptional de-repression in the *adcp1* mutant. In this model, transposon upregulation could lead to the accumulation of activating marks, such as H3K4me3 or histone acetylation, which could interfere with H3K9me2 binders such as SHH1 (required for maintenance of RNA-directed DNA methylation) or CMT2/CMT3, which would ultimately lead to losses of H3K9me2 because of the epigenetic feedback loops.[1] This model predicts that H3K9me2 losses at specific loci would be correlated with transcriptional upregulation in *adcp1*, something that was not formally tested by either Zhao or Zhang and colleagues. However, it is worth noting that the transcriptional changes reported by both groups were modest, with relatively few differentially expressed transposons identified. A third possibility, proposed by the authors, is that ADCP1 may stimulate the formation of phase transitioned nuclear condensates that promote the recruitment of chromatin regulators such as SUVH4/5/6 or CMT2/CMT3 to make these feedback loops operate more efficiently.

A final perspective to consider is how ADCP1 promotes silencing of transposons. ADCP1 could either recruit, or itself act, as a repressor protein. While ADCP1 does not encode known repressor domains, it does encode three tandem Agenet domains, each with the ability to bind H3K9me2. Zhang and colleagues showed that ADCP1 binds H3K9me2 with a stoichiometry of 1:3, and thus may bind adjacent nucleosomes causing transcriptional repression by promoting chromatin compaction (Fig. 1). In terms of repressor protein recruitment, the IP-MS of ADCP1 performed by Zhang and colleagues did not reveal any obvious candidates. Loss of silencing in *adcp1* could also simply be a secondary consequence of loss of DNA and histone methylation, which would be consistent with the proposed role of ADCP1 in promoting a chromatin environment conducive to the optimal functioning of chromatin regulators.

ADDITIONAL INFORMATION

Conflict of interest: The authors declare that they have no conflict of interest.

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