Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

The seekers: how epigenetic modifying enzymes find their hidden genomic targets in *Arabidopsis*

Xian Deng[1], Qi Qiu[1], Kaixuan He[1,2] and Xiaofeng Cao[1,2]

Epigenetic regulation plays fundamental roles in modulating chromatin-based processes and shaping the epigenome in multicellular eukaryotes, including plants. How epigenetic factors recognize their target loci hiding in the vast genomic DNA sequence remains a long-standing mystery. During the past several years, a growing body of work has revealed the complex, dynamic, and diverse chromatin-targeting mechanisms of these epigenetic factors. In this review, we focus on recent advances in understanding the recruitment of epigenetic factors to specific genomic regions, based on data from *Arabidopsis thaliana*.

Addresses
[1] State Key Laboratory of Plant Genomics and National Center for Plant Gene Research, CAS Center for Excellence in Molecular Plant Sciences, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China
[2] University of Chinese Academy of Sciences, Beijing, China

Corresponding author: Cao, Xiaofeng (xfcao@genetics.ac.cn)

Current Opinion in Plant Biology 2018, **45**:xx–yy

This review comes from a themed issue on **Cell signalling and gene regulation**

Edited by **Jorge Casal** and **Javier Palatnik**

https://doi.org/10.1016/j.pbi.2018.05.006

Introduction

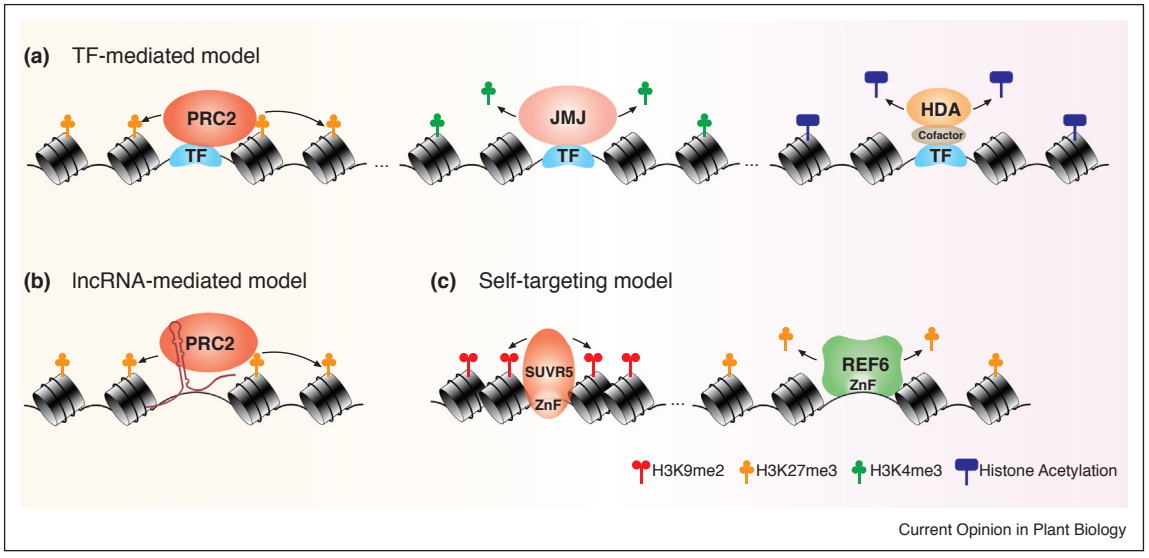
Epigenetic regulation of gene expression is finely modulated by diverse molecular events, including DNA methylation, histone modifications and variants, chromatin remodeling, and alterations in noncoding RNA profiles [1,2]. Epigenetic control has indispensable functions in multiple aspects of development and cellular processes of multi-cellular organisms [2,3]. Many enzymes modify DNA and histones in epigenetic regulation and their functions require targeting or binding to the target chromatin loci, and subsequent triggering of the enzymatic activity [4]. Emerging studies using multidisciplinary approaches have described genome-wide epigenetic profiles [2,5]; however, a long-standing mystery in epigenetic regulation is how these epigenetic factors recognize their genomic loci in the vast DNA sequence of the genome. The establishment of epigenetic profiles relies on the recruitment of chromatin modifiers, in either a chromatin state-dependent manner or a sequence-specific manner [6,7]. Comprehensive and detailed reviews have examined the sequence specificity of DNA methylation in mammals [8] and in plants [9]. Here we focus on recent advances illuminating the fundamental molecular mechanisms by which histone modifiers recognize specific DNA sequences in the genome in *Arabidopsis*. Understanding the sequence-specific targeting mechanism of these histone modifiers is an important stepping stone towards mechanistic insight into the complexity of the epigenetic landscape. Specifically, we examine targeting mechanisms mediated by transcription factors, long non-coding RNAs, and the enzyme itself (Figure 1).

Transcription factor-mediated targeting

Transcription factors (TFs) bind to DNA in a sequence-specific manner and regulate gene expression by promoting or suppressing transcription, thus ensuring appropriate levels of gene expression, in certain temporal and spatial patterns [10,11]. Although the roles of epigenetic factors and TFs in transcription remain a subject of active debate, they usually act together to regulate gene expression and cell fate determination, as sequence-specific DNA-binding TFs direct epigenetic factors, as well as other transcriptional cofactors, to the target chromatin regions [12].

Polycomb repressive complex 2 (PRC2)-mediated trimethylation of histone H3 lysine 27 (H3K27me3), a facultative repressive epigenetic mark for chromatin compaction, has a key role for cell identity and developmental regulation in multicellular eukaryotes [13,14,15•]. However, how PRC2 recognizes its target loci is a long-standing key question. In *Drosophila*, PRC2 is recruited to Polycomb response elements (PREs), the specific *cis*-regulatory sequences with variable length around gene promoters [16,17]. Multiple TFs act as intermediary to interact with PRC2 on the one hand, and recognize specific *cis*-elements within PREs on the other hand, which are needed for H3K27me3 spreading and gene repression [18]. In *Arabidopsis*, specific sequence elements have been identified to be required for PRC2 recruitment and H3K27me3 deposition on individual target loci, which are involved in diverse developmental stages [19]. These PRE-containing target genes include *AGAMOUS* [14], *PHERES 1* [20], *SEPALLATA 3* [21], *LEAFY COTYLEDON 2* [22], *WUSCHEL* [23], *BREVIPEDICELLUS*, *KNAT2* [24], *KNUCKLES* [25], *ABSCISIC*

Figure 1



Mechanisms regulating sequence-specific targeting of histone modifiers on chromatin. **(a)** TF-mediated targeting mechanism. Some histone modifiers are targeted to specific genomic target loci through interaction with sequence-specific DNA-binding transcription factors and/or cofactors. **(b)** Non-coding RNA-mediated targeting mechanism. Some histone modifiers are targeted to specific genomic regions through interaction with lncRNAs. **(c)** Self-targeting mechanism. Some histone modifiers interact with chromatin via their own DNA binding domains.

ACID INSENSITIVE4 [26], and *FLOWERING LOCUS C* [27••,28••]. In addition, some consensus sequence motifs, such as the *GAGA* and *telobox cis*-elements, were reported to be involved in PRC2 recruitment [29–32]. These studies, however, are either limited to case-by-case tests or lack genetic evidence. Two recent studies uncovered the consensus PREs and sequence-specific TFs (including C2H2-ZnF, AP2-ERF, BBR-BPC, and TRBs) that are responsible for PRC2 recruitment in *Arabidopsis*, providing an evolutionarily conserved model, similar to that described in *Drosophila* [33••,34••]. They found that different TFs interact with diverse subunits of PRC2 complex, reflecting a complex mode of recruitment [33••,34••]. There are several PRC2 complexes, for example, EMF, VRN, and FIS complexes, regulating various biological aspects of plants owing to gene duplication of the PRC2 subunits, so it will be interesting to identify the combinations of PRE-TFs-PRC2 interaction during plant development. Since PREs evolve rapidly in different species [35], it will be interesting to identify PREs and TFs responsible for PRC2 recruitment in other plant species, and study whether and to which extent PREs and TFs are conserved among different plant species. Furthermore, natural variation may affect the composition of PREs and the ability to recruit PRC2 in different *Arabidopsis* ecotypes, which might add regulatory complexity to this model.

Some histone demethylases require TFs to target certain chromatin loci, in mammals [[47•]] and in plants [36,37•]. In mammals, for instance, the zinc finger protein ZNF711 binds an H3K9me2/me1 demethylase, PHF8, and recruits PHF8 to a subset of target genes involved in X-linked mental retardation [38]. *Arabidopsis* JUMONJI 14 (AtJMJ14), a Jumonji C (JmjC)-domain-containing Lysine (K)-Specific Demethylase 5 (KDM5) subfamily member, has H3K4me3 demethylase activity that regulates flowering time [39–41], shoot regeneration [42], and RNA-mediated gene silencing [43–46]. AtJMJ14 directly interacts with two closely related DNA sequence-specific NAC (NAM, ATAF, CUC) domain-containing transcription factors, NAC050 and NAC052, and is subsequently targeted to certain $CTTG(N)_5CAAG$ containing NAC050/052-binding chromatin regions [36,37•]. To further investigate how AtJMJ14 specifically recognizes H3K4me3, the crystal structure of the AtJMJ14 catalytic domain was analyzed, revealing the essential role of conserved acidic residues in the substrate selectivity [47•]. This study indicates a common substrate recognition mechanism for KDM5 subfamily demethylases, and provides insight into targeted design for cancer treatment [47•].

In addition to direct interaction, transcription factors can recruit histone modifiers to target loci via cofactors. Histone deacetylase 6 and 19 (HDA6 and HDA19) are two closely related deacetylases that facilitate transcriptional repression in plants [48]. HDA6 and HDA19 are recruited to the specific genomic regions through ethylene-responsive element binding factor-associated amphiphilic repression (EAR) motif-containing TFs, via direct interaction of TFs with co-repressors, such as SIN3-associated