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Plant homeodomain fingers form a helping hand for transcription

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Abbreviations: PHD, plant homeodomain; PHF2/8, plant homeodomain finger 2/8; JmjC, jumonji carboxyterminal domain; XLMR, X-(chromosome) linked mental retardation; PTM, post-translational modification; KMT, histone lysine methyl-transferase; SET, *Su(var)*3-9/enhancer of zeste/trithorax domain; MLL, mixed lineage leukemia; KDM, histone lysine demethylase; PRC, polycomb repressive complex; ChIP-seq, chromatin immunoprecipitation followed by deep sequencing; ING, inhibitor of growth; ES-cells, embryonic stem cells; RNAPI/II, RNA polymerase I/II

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Several recent publications demonstrate a co-activator function for a subgroup of plant homeodomain fingers, which, in humans, comprises PHF2, PHF8 and KIAA1718. Besides an N-terminal plant homeodomain (PHD), these proteins also harbor an enzymatically active Jumonji-C domain (JmjC). While they have been shown to bind via their PHDs to H3K4me3-bearing nucleosomes at active gene promoters, their JmjC-domains are able to remove mono- and dimethyl-lysine 9 or 27 on histone H3 or monomethyl-lysine 20 on histone H4, chromatin modifications that correlate with transcriptional repression. Such dual histone crosstalk ensures the proper removal of repressive histone marks following transcriptional activation by RNA polymerases I and II. Mutations in the *PHF8* gene lead to X-linked mental retardation (XLMR) and knockdown of KIAA1718 and PHF8 homologs in zebrafish causes brain defects. Thus, the co-activator function of this new class of chromatin-modifying enzymes has important functional roles in neuronal development. To continue with the nomenclature for histone demethylases, we propose the usage of KDM7A, -B and -C for KIAA1718, PHF8 and PHF2 proteins, respectively.

Histone Modifications and Chromatin-Modifying Enzymes

A host of transcriptional co-activators and co-repressors function through post-translational modifications (PTMs) of histones.[1] An important and well-studied type of PTM in this respect is methylation of the ε-aminogroup of the lysine sidechain, which can occur in three possible states, namely mono-, di- or trimethylation (me1/2/3, respectively). Depending on the histone variant, the position of the lysine and its methylation state, such modifications may signal different outcomes. For example, trimethylation at lysines 4, 36 and 79 of histone H3 (H3K4me3, H3K36me3, H3K79me3) are positively correlated with transcription and thus considered as activating chromatin marks. While H3K4me3 is strongly enriched at proximal promoters, H3K36me3 and H3K79me3 are found throughout transcribed gene bodies.[2] Conversely, di- and trimethylation at lysines 9 and 27 of histone H3 (H3K9me3/2, H3K27me3/2) spread on silent chromatin and therefore are often correlated with transcriptional repression.

Methyl-marks are deposited on lysine residues by protein complexes that contain lysine methyl transferase enzymes (KMTs), most of which contain a *Su(var)*3-9/Enhancer of Zeste/Trithorax (SET) domain. For instance, in humans H3K4-methylation is conferred by enzymes of the KMT2-family (SET1A/B and mixed lineage leukemia MLL1-5).[3] The latter are thought to be recruited by transcription factors upon gene induction and to methylate nucleosomes at the respective promoters. Subsequently, a diverse number of proteins recognizing H3K4me3 come into play, some of which have already been shown to stimulate transcription (e.g., TAF3, BPTF).[4,5]

On the other hand, methyl-groups can be removed from lysines by histone demethylase enzymes (KDMs) containing either flavin-dependent amine-oxidase or Fe^{2+} and α-ketoglutarate-dependent Jumonji-C (JmjC) domains.[6]

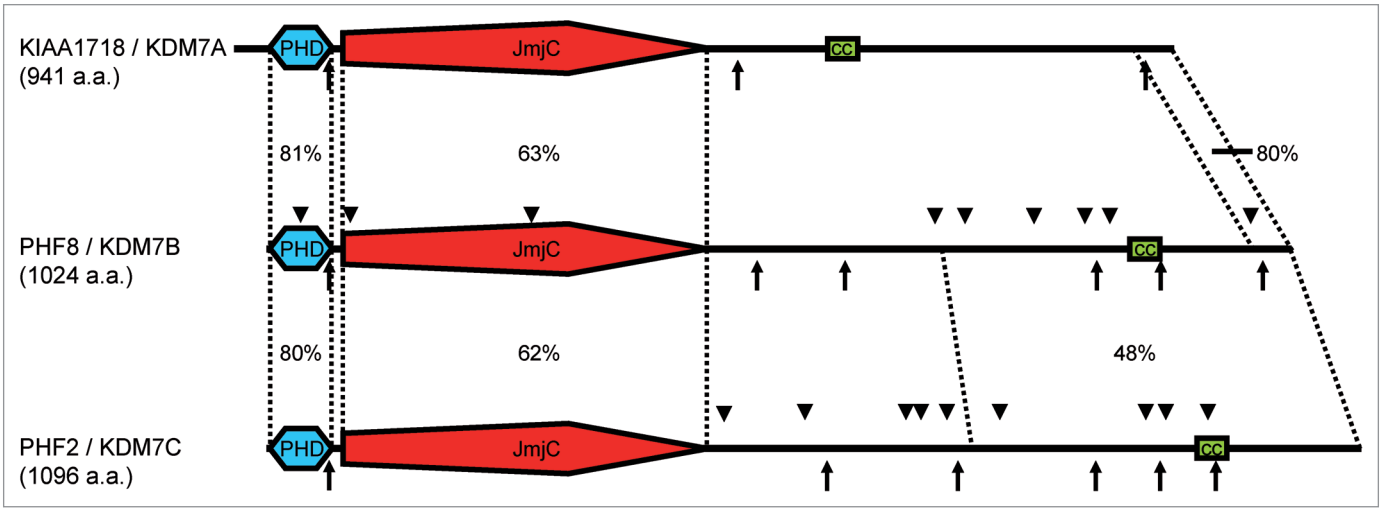

Figure 1. KDM7-family proteins. In humans the KDM7-family comprises three members: KIAA1718, PHF8 and PHF2. These proteins of about 1,000 amino acids (a.a.) contain an N-terminal plant homeodomain (PHD), a Jumonji C-domain (JmjC) and a short coiled coil region (cc). Nuclear localization signals and phosphorylation sites are depicted as arrows below and arrowheads above the model, respectively. Homology between the family members is given in percent identity for PHD, JmjC-domain and C-terminal region defined by dashed lines.

The aforementioned methylation of lysine 4 on histone H3 can be removed by either LSD1/KDM1- or JARID1/KDM5-family histone demethylases.[3] Intriguingly, these KDMs can be components of polycomb repressive complexes (PRCs) that synergize to bring about transcriptional repression.[7,8] Interestingly, in addition to their catalytic domains most chromatin-modifying enzymes possess additional chromatin and/or DNA-binding modules.[9] Such modular nature of chromatin-modifying enzymes allows for additional specificity in their recruitment and/or mode of action.[10] Therefore, it is likely that recruitment of such enzymes to their target is accomplished by multiple synergistic interactions of the components of the chromatin-modifying complex and different modifications of the chromatin. Such a mechanism would allow regulation of specificity as well as affinity by tissue-specific variation of subunit composition combined with dynamic changes in histone post-transcriptional modifications allowing for fine-tuning of gene expression in a tissue- and time-specific manner.

The Plant Homeodomain (PHD): A Chromatin Reader Module

Recently, we and others found a new example of crosstalk between histone modifications.[11-17] A class of JmjC-domain chromatin-modifying enzymes is characterized by a single N-terminal plant homeodomain zinc finger (PHD), a domain that was shown to associate with methylated lysine residues.[18] In humans this group consists of three members: the plant homeodomain fingers 2 and 8 (PHF2 and PHF8) and KIAA1718 (**Fig. 1**). Structures of their PHDs and JmjC-domains have now been solved by X-ray crystallography.[13,15,19-21] Besides conserved zinc-chelating residues, these PHDs comprise a patch of phenylalanine and tyrosine residues called aromatic cage, which can occur in several domains and can interact with methylated lysine. Biochemical experiments demonstrated that the three proteins interact specifically with histone H3 methylated at lysine 4 via their PHDs.[11-15,22] Mutation of aromatic cage amino acids abolishes this interaction. Binding assays with doubly modified histone H3 peptides indicate that most adjacent PTMs do not affect the interaction between H3K4me3 and the PHD of PHF8/KDM7B, but that phosphorylation of threonine 3 does, a modification mediated by the mitotic kinase haspin.[12,23] Additionally, phosphorylation of PHF8 at serines 33 and 84 abolishes its binding to mitotic chromosomes.[16] However, during interphase, PHF8 is present at thousands of gene promoters in several cell lines as revealed by chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) using different cell lines and antibodies.[12,14,16,17] Similar results were obtained for F29B9.2/KDM7A in *C. elegans*.[24] Thus, we assume that the localization is mainly determined by binding of the PHD to H3K4-trimethylated nucleosomes, and that PHF2/KDM7C has similar properties. Nevertheless, about 30% of highly H3K4me3-enriched promoters are not co-occupied by PHF8, which indicates that there must be other factors influencing the recruitment.[12,14,16]

Furthermore, there are exceptions to the rule that H3K4me3 is generally linked to active gene expression. For example, a class of PHD-containing proteins, the inhibitor of growth family (ING1-5), has been demonstrated to bind to H3K4me3 but to repress transcription upon DNA-damage.[25] On the other hand, the PHD-protein AIRE was shown to interact with unmethylated lysine 4 on histone H3, leading to transcriptional activation.[26] Additionally, there is a class of poised chromatin regions in mouse embryonic stem cells known as bivalent domains, which harbor both H3K4me3 and H3K27me3 chromatin modifications. In embryonic stem (ES) cells many developmental genes bear bivalent domains and are thought to be rapidly induced by removal of such repressive chromatin modifications upon

Table 1. Human KDM-families with JmjC-domains

JmjC-enzyme(s)	Substrate(s)	Conserved Fe^{2+}/α-KG binding a.a.	Function
KIAA1718 (KDM7A) PHF8 (KDM7B) PHF2 (KDM7C)	H3K27me2/1; H3K9me2/1 H3K9me2/1; H4K20me1 H3K9me1	H D H/T K H D H/T K H D Y/T K	activators
JHDM1A/B (KDM2A/B)	H3K36me2/1	H D H/T K	repressors
JHDM2A/B (KDM3A/B)	H3K9me2/1	H D H/T K	activators
JHDM3A-D (KDM4A-D)	H3K9me3/2; H3K36me3/2	H E H/F K	activators
JARID1A-D (KDM5A-D)	H3K4me3/2	H E H/F K	repressors
UTX (KDM6A) JMJD3 (KDM6B)	H3K27me3/2 H3K27me3/2	H E H/T K H E H/T R	activators

α-KG, α-ketoglutarate; a.a., amino acids

differentiation.[27] This may be accomplished by displacement of PRCs with activating complexes that exhibit demethylase activity towards repressive marks.

The Jumonji C-Terminal Domain (JmjC) Confers Demethylation

Active JmjC-domains comprise several conserved amino acids that are needed for correct binding of the crucial cofactors Fe^{2+} and α-ketoglutarate (JmjC-containing KDM-families are listed in **Table 1**). Besides those, the KDM7-family displays four short additional α-helices in the carboxyterminal region of the JmjC which are essential for activity. The described enzymes act on methylated H3K9, H3K27 and/or H4K20. Interestingly, H3K9 and H3K27 are placed in a similar context (-ARKS-), while H4K20 is not (-HRKV-). Activity has been demonstrated by in vitro assays using peptides, bulk histones or nucleosomes as substrates and in vivo by overexpression and/or knockdown experiments.[11-17,19,20,22,28,29] While demethylation assays with peptides also stated marginal activity towards H3K36me2, there is little evidence that these enzymes remove this histone modification in vivo. The three new members exhibit slightly different specificities (**Table 1**). While PHF8 preferentially acts on H3K9me2/1 and H4K20me1, KIAA1718 mainly demethylates H3K9me2/1 and H3K27me2/1.[13,16,17,22,28] Since the active centers cannot accommodate trimethylated lysines these do not constitute viable substrates for these enzymes. Intriguingly, JmjCs that exhibit aspartate as Fe^{2+}-chelating residue seem to be specific for me2/1, while glutamate at this position confers specificity for me3/2 (**Table 1**). In contrast, thus far PHF2 was only shown to demethylate H3K9me1.[15] The difference might be due to the fact that this protein, instead of the second conserved Fe^{2+}-binding histidine, contains a tyrosine residue at this position. Trimethylation of H3K4 in cis greatly enhances PHF8 demethylase activity toward H3K9me2.[11,13] However, while PHF8 can mediate H3K4me3-binding through its PHD domain and H3K9me2-demethylation on the same histone tail as a result of its flexible linker between PHD and JmjC, KIAA1718 has a rigid linker and an extended N-terminus, precluding it from demethylation of H3K9me2 once its PHD associates with H3K4me3.[13] When H3K4me3 peptides are added to H3K9me2-demethylation reactions in trans KIAA1718 is inhibited and PHF8 is not stimulated anymore.[11,13] Nevertheless, in vivo H3K4me3 might as well stimulate demethylation of H3K9me2/1, H3K27me2/1 and H4K20me1 on neighboring histone tails because it anchors the KDM7 enzymes in close proximity.[21] This possibility may explain why H4K20me1 demethylation could only be detected on nucleosomal substrates but not on core histones.[16,17] Obviously, H3K4me3-marks and a functional PHD stimulate demethylation by overexpression of PHF8 in vivo.[12] Consequently, chromatin-association of PHF8 via the interaction between PHD and H3K4me3 is crucial for proper function. On the other hand, though mutations of cofactor binding residues in the JmjC render PHF8 catalytically inactive, it still retains most of its co-activation capacity, at least in episomal reporter assays.[12] Thus, we expect that additional protein interactions are of particular importance for proper function of KDM7 family members.

The Carboxyterminal Half and Interaction Partners

Little is known about the properties of the C-terminal halves of KDM7 proteins. They do not contain any known protein domains, and the homology between the three human proteins is low in this region compared to that of PHD and JmjC (**Fig. 1**). However, we found that direct association of PHF8 with the carboxyterminal domain of RNA polymerase II (RNAPII) largest subunit strongly depends on this part of the protein.[12] Other described interactions of PHF8 with RNA polymerase I (RNAPI), KMT2-complex components, HCF-1, E2F1, ZNF711 and RAR may also be mediated by its C-terminal part (**Fig. 2**).[11,14,16,30] The KDM7 C-termini contain nuclear localization signals suggesting their importance for correct localization (**Fig. 1**). Additionally, all three human proteins contain a putative coiled coil region (**Fig. 1**). PHF2 and PHF8 also exhibit several phosphorylation sites which appear to be important for the regulation of their activity (**Fig. 1**).[16]

Biological Function and Target Genes

Recent findings point to the KDM7-family of histone demethylases as a new class of transcriptional co-activators. By virtue of association with H3K4me3- and removal of H3K9me2/1-, H3K27me2/1- or H4K20me1-modifications, KDM7

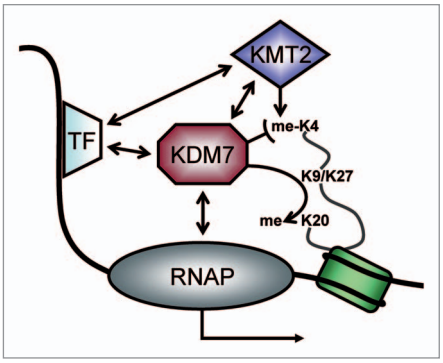

Figure 2. Simplified model of action for transcriptional co-activation by KDM7-proteins. Upon gene induction, transcription factors (TF) bind to specific DNA-sequences and recruit co-activators such as H3K4-methyltransferases (KMT2). The latter introduce methyl-marks on histone H3 tails (me-K4) which protrude from nucleosomes. These activation chromatin marks are bound by proteins such as histone demethylases of the KDM7-family. This class of chromatin-modifying enzymes removes repressive methyl-marks from K9 or K27 on H3 or K20 on H4. Furthermore KDM7 can interact with RNA polymerases I or II (RNAP) and other chromatin-associated factors (such as E2F1 and ZNF711). Both mechanisms lead to the activation of transcription. However, the transcription factors and interactions may be specific for cell type, time point and gene locus, which could be the means by which KDM7-activity is regulated.

proteins create a more permissive chromatin environment at promoters (**Fig. 2**). Only then the respective demethylated lysine residues can be targeted for acetylation by acetyltransferases, which may lead to enhanced expression. Moreover, additional interactions with RNAPI, RNAPII and other proteins may further stimulate transcription and could play a role in the regulation of activity. Furthermore, other unknown (non-)histone proteins could be substrate for binding and/or demethylation by KDM7-members and nascent RNA could be involved in this process as well.[31]

Importantly, mutations in the *PHF8* gene can cause Siderius-Hamel syndrome, an X-linked mental retardation (XLMR), which is often accompanied by cleft lip and/or cleft palate.[32] The mutations mostly lead to truncations before or in the JmjC domain; however, a complete deletion of the locus and a point mutation has also been described.[33-36] These aberrations cause loss of function as demonstrated for the XLMR point mutant F279S, which mislocalizes probably due to misfolding of the JmjC domain. Depletion of PHF8 does not result in a global increase of histone methylation but in a rather modest increase of H4K20me1 and H3K9me1-levels at target promoters.[16,17] Surprisingly, H3K9me2, H3K27me2 and H3K36me2 remain mostly unchanged. In contrast, loss of one of the two homologs in *C. elegans* (F29B9.2) leads to global increase in H3K9me2 as well as H3K27me2 and affects locomotion.[14] In addition, RNA interference-mediated depletion of PHF8- or KIAA1718-homologs disturbs neuronal differentiation of mouse ES-cells and causes defects in brain and craniofacial development in zebrafish.[17,22,28,30] Liu et al. suggests that the loss of PHF8 affects cell cycle progression due to impaired co-activation of E2F1 targets.[16] However, the specific defects of XLMR patients in neuronal and midline development may not be a reflection of the general function of PHF8 in growth but rather result from a specific role PHF8 may play in neuronal proliferation.[17] PHF8 function could be critical for neurons as its activity may not be compensated by other family members in neuronal cells or that it may have neuronal-specific targets.

PHF8 and PHF2 both play a role as co-activators of ribosomal RNA transcription where they might act redundantly.[11,15,37] Though knockdown of PHF8 results in downregulation of several RNAPII-transcribed genes, changes in histone methylation and expression levels are subtle and there is little overlap between targets found in different studies.[12,14,16,17] Interestingly, one candidate target gene, *SMCX* (also known as *JARID1C* or *KDM5C*) codes for another KDM linked to XLMR, which also contains PHDs and a JmjC domain. In contrast to KDM7, KDM5C demethylates H3K4me3/2, binds to H3K9me3 and acts as a transcriptional repressor.[8] Overexpression of other candidate targets like FGF4, FST and MSX1 can in part rescue the loss of KDM7 proteins in model systems.[17,22,28]

Taken together, it is not clear whether the *PHF8* mutations leading to impairment of transcription by RNAPI, RNAPII or both accounts for manifestation of XLMR. The gene expression changes are rather subtle and therefore it is possible that a combined decrease in transcriptional output of multiple targets may be the underlying cause of the disease phenotype. Further experiments using model organisms or XLMR-patients' material will shed additional insights on the target genes and biological functions of the KDM7-family members.

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