Published in final edited form as:

Biochem J. ; 476(16): 2351–2354. doi:10.1042/BCJ20190492.

The PHD finger of Spp1 mediates histone modification cross-talk

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Abstract

Binding of the Spp1 PHD finger to histone H3K4me3 is sensitive to adjacent post-translational modifications in the histone tail. This commentary discusses the findings of He and colleagues [*Biochem. J.* **476**, 1957–1973] which show that the PHD finger binds to H3K4me3 in a selective manner which is conserved in the *Saccharomyces pombe* and mammalian orthologues of Spp1.

The eukaryotic genome is packaged in the cell nucleus in the form of chromatin, a complex of DNA and histone proteins. Chromatin allows for tight compaction of the genome and through changes in its structure controls DNA accessibility necessary for many fundamental cellular processes such as gene transcription and DNA replication, recombination and repair. How the chromatin landscape is regulated to achieve the proper genomic state for each process remains an open question in the field. The presence of post-translational modifications (PTMs) on histone proteins has emerged as one of the major mechanisms for altering chromatin structure and function. A large number of histone PTMs have been identified that either directly impact histone–DNA or histone–histone interactions or mediate binding of chromatin-associated proteins (CAPs) to DNA and histones. Importantly, PTMs are found to not only act in a solitary fashion but also often work in concert with other PTMs and/or influence each other's activities. For example, a single PTM may be capable of being recognized by two CAPs, but a second modification can promote the association of one of these CAPs while inhibiting the association of the second CAP, hence leading to selectivity (Figure 1). This type of interplay, also named PTM cross-talk, is largely mediated by domains, known as reader domains or simply readers within the CAPs, that recognize the histone PTMs.

In the paper published in the recent issue of *Biochem. J.*, He et al. [1] characterize the association of a CAP called Spp1 with histone H3 and investigate its role in mediating histone PTM cross-talk. Spp1 is a *Saccharomyces cerevisiae* protein which is conserved through mammals, where it is called CFP1 [2]. Spp1 is a subunit of the methyltransferase COMPASS (complex proteins associated with Set1) complex that catalyzes histone H3K4

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

methylation, producing the H3K4me mark. Intriguingly, Spp1 recognizes H3K4me and enhances the activity of this complex. In addition, Spp1 is involved in meiotic recombination, where it tethers Mer2 to H3K4me-enriched chromatin leading to the double-strand break formation necessary for initiation of the recombination process. The N-terminus of Spp1 contains the plant homeodomain (PHD) finger, a reader domain that most often binds unmethylated H3K4 (H3K4me0), di-methylated H3K4 (H3K4me2), or tri-methylated H3K4 (H3K4me3). It has been proposed that it is this region that leads to Spp1 association with methylated H3K4 and thus allows the COMPASS complex and Mer2 to respond to this histone PTM. In the current study, He et al. confirm the presence of the PHD finger fold and find that it needs an adjacent C3H zinc finger for stability. The authors further show that the PHD finger selectively binds to H3K4me3, and that this selectivity is conserved in the *Saccharomyces pombe* and mammalian orthologues of Spp1, though the absolute affinity for H3K4me3 differs between the proteins.

The structure of the *S. cerevisiae* Spp1 region containing the PHD finger and the adjacent C3H zinc finger was obtained in complex with an H3K4me3 peptide. The way in which the Spp1 PHD finger binds to the methylated histone tail is highly reminiscent of binding of the other known methyllysine-recognizing PHD fingers found in various CAPs [3–5]. The side chain of trimethyllysine occupies an aromatic cage of the Spp1 PHD finger and is restrained through cation- π and hydrophobic interactions within the cage, but one of the walls of the cage is made of an aspartate residue. The presence of the negatively charged aspartic or glutamic acid in the aromatic cages of some methyllysine-recognizing readers (such as Tudor and MBT) leads to selectivity for mono- or dimethylated lysine. Despite the presence of aspartate in the binding pocket of the Spp1 PHD finger, this reader shows some preference for H3K4me3 over H3K4me2, behaving similarly to the PHD fingers of MLL5, SET3 and TAF3 that also contain an aspartate in their aromatic cages and select for H3K4me3 [6–8]. The inability of an aspartate in the PHD fingers to be engaged with the dimethylammonium moiety might arise from the rigid position of the aspartate, whose side chain is constrained by hydrogen bonds with either PHD finger residues (the backbone amide of Gly33 in the case of Spp1), or with T6 of H4K4me3 in other PHD finger complexes. These results also suggest that the shorter aspartic side chain in the PHD finger's aromatic cage does not provide selectivity for the low-methylation states of H3K4, and a longer glutamic side chain is needed to select for H3K4me2, as in the native PHD finger of PHF20 [9] or the engineered PHD finger of BPTF [10].

In addition to caging the trimethylated lysine of H3K4me3, the Spp1 PHD finger makes contact with several other histone residues. The extra contacts provide sensitivity to adjacent histone PTMs, allowing for the PHD finger to mediate specific histone PTM cross-talk. In particular, methylation of H3R2 and phosphorylation of H3T6 weaken the interaction with H3K4me3, and phosphorylation of H3T3 completely disrupts the interaction. Moreover, He et al. show that this sensitivity is conserved in the orthologous *S. pombe* and mammalian proteins. These patterns of histone PTM cross-talk are very similar to the patterns observed for other PHD fingers, several of which have been found to be sensitive to modifications of H3R2, H3T3, H3T6, or some combination of these PTMs [11–13]. However, the extent of sensitivity to a PTM by any given PHD finger is distinct. In other words, for an individual PHD finger, the effect of H3R2, H3T3, or H3T6 modification on the interaction with

H3K4me will vary as compared with another PHD finger, and this selectivity is crucial in allowing different CAPs to uniquely respond to and associate with specific chromatin states. Intriguingly, phosphorylation of H3T3, which completely disrupts Spp1 PHD finger binding to H3K4me3 is important in meiotic resumption, indicating that this may act as a switch to disengage the Spp1/CFP1 PHD finger during meiosis. In contrast, phosphorylation of T6 and methylation of R2, which only weaken binding to H3K4me3, may act more to fine-tune the interaction with H3K4me3. Together, these results highlight that while the basic mechanism of histone binding is highly conserved, each PHD finger is unique in its selectivity and ability to mediate histone PTM cross-talk. An important question that now arises is how all of these PHD finger containing proteins relate to each other, and how their unique selectivities reflect on the biological outcomes.

A final notable aspect of this study is in the structural approach itself. Recently, there has been a revolution in the field of cryo-electron microscopy (cryo-EM) allowing for structures of large complexes to be determined at a substantially higher resolution than ever seen before. Moreover, there has even been the suggestion that other structural biology approaches such as the X-ray crystallography approach used in this paper may no longer be needed in the near future. In fact, the structure of the COMPASS complex has recently been determined by cryo-EM to 4 Å resolution [14]. However, though the C-terminal half of Spp1 in the cryo-EM structure resolved well, the N-terminal half including the PHD finger and C3H domain did not resolve in the structure, and this deficiency has also been seen in cryo-EM structures of other reader-containing CAPs. Thus, this work exemplifies the strong need to keep merging more than one structural biology approach for a full characterization of these important biological systems as they are often dynamic and involve multiple but relatively weak interactions.

Acknowledgements

Research in the Musselman and Kutateladze laboratories is funded by the NIH.

Abbreviations

CAPs	chromatin-associated proteins
COMPASS	complex proteins associated with Set1
cryo-EM	cryo-electron microscopy
PHD	plant homeodomain
PTMs	post-translational modifications

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Figure 1. Histone PTM cross-talk.

Two chromatin-associated proteins (CAP1 and CAP2) can both associate with a nucleosome containing a single PTM (left, top and bottom). However, a second modification can promote the association of one of these CAPs (CAP1, top right) while inhibiting the association of the second CAP (CAP2, bottom right), leading to selectivity.