Mini-Review

Diverse roles of WDR5-RbBP5-ASH2L-DPY30 (WRAD) complex in the functions of the SET1 histone methyltransferase family

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WD repeat containing protein 5 (WDR5), Retinoblastoma Binding Protein 5 (RbBP5), Absent-Small-Homeotic-2-Like protein (ASH2L), and Dumpy-30 (Dpy30) have been reported to be the integral and shared components of all the SET1 family of histone 3 lysine 4 histone methyltransferase (HMT) complexes. Collectively called the WRAD complex, these proteins are pivotal to the HMT activity of the SET1 complexes. Recent reports highlight the novel non-canonical functions of WRAD in cellular processes other than its well-studied role in histone methylation and gene expression. In this review, we examine the diversity in emerging transcription-independent functions of WRAD.

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1. Introduction

Post-translational histone modifications add another layer of regulation to the ever changing chromatin landscape to activate or repress transcription. N- or C-terminal tails of histones can be methylated, phosphorylated, acetylated, ubiquitinated and, even, sumoylated or ribosylated to impact gene expression. All these histone modifications recruit chromatin remodelling protein complexes or alter the structure of chromatin (Bannister and Kouzarides 2011). In particular, lysine methylation regulates the activation (H3K4, H3K36, and H3K79) as well as repression (H3K9, H3K27, and H4K20) of transcription by methylation of specific residues in the histones (reviewed in Martin and Zhang 2005). Histone 3 Lysine 4 methylation (H3K4me) is closely associated with the activated state of the chromatin (Martin and Zhang 2005; Eissenberg and Shilatifard 2010). In mammals, the SET1 family of histone methyltransferases (HMT) is responsible for depositing the H3K4 methylation mark on promoters of active genes (Ernst and Vakoc 2012; Piunti and Shilatifard 2016).

The SET1 family include at least six multi-protein complexes namely Mixed Lineage Leukemia (MLL1 or MLL), MLL2, MLL3, MLL4, Set1A, and Set1B proteins (Cao 2012; Ernst and Vakoc 2012). All these enzymes have unique non-redundant roles (Ansari and Mandal 2010; Shilatifard 2012; Shinsky and Cosgrove 2015). While Set1A and Set1B impart H3K4me3 mark to majority of gene promoters in mammals, MLL1 has been shown to regulate a subset of genes important during development, including the *Hox* loci (Wu *et al.* 2008). In contrast, MLL3 and MLL4 are involved in depositing the H3K4 mono-methylation mark at the enhancers (Herz *et al.* 2012; Hu *et al.* 2013). Like other methyltransferases, all members from this family act as multi-protein complexes with common subunits namely, WD repeat containing protein 5 (WDR5), Retinoblastoma Binding Protein 5 (RbBP5), Absent-Small-Homeotic-2-Like protein (ASH2L) and Dumpy-30 protein (DPY30) (Dou *et al.* 2006; Steward *et al.* 2006; Takahashi *et al.* 2011; Ernst and Vakoc 2012; van Nuland *et al.* 2013). Additional context-specific subunits also associate to impart unique properties to the complex (Yokoyama *et al.* 2004; Cho *et al.* 2007; Wu *et al.* 2008; Patel *et al.* 2007, 2014).

2. The conventional functions of WRAD

The WDR5, RbBP5, ASH2L, and DPY30 collectively called WRAD, along with the Set1/MLL Su(var)3-9,

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Enhancer-of-zeste, Trithorax (SET) domain form a minimal 'core complex' capable of optimal enzymatic activity (Dou *et al.* 2006; Steward *et al.* 2006; Patel *et al.* 2011; Ernst and Vakoc 2012; Dharmarajan *et al.* 2012; Shinsky and Cosgrove 2015; Zhang *et al.* 2015). *In vitro* reconstitution studies with the purified WRAD proteins and the catalytic SET domain of MLL have provided deeper insights into the role of WRAD in the functions and the assembly of the MLL complex (Steward *et al.* 2006; Odho *et al.* 2010; Dharmarajan *et al.* 2012; Senisterra *et al.* 2013). *In vitro*, MLL by itself is a mono-methyltransferase (Patel *et al.* 2008b; reviewed in Cosgrove and Patel 2010). However in association with WRAD, it is capable of di- and tri-methylation activities (Zhang *et al.* 2015). While WDR5, RbBP5, and ASH2L form the minimal WRA complex required for the methylation activity observed with the WRAD complex, DPY-30 increases the catalytic efficiency and specificity of WRAD for histone H3 peptide (Cosgrove and Patel 2010; Patel *et al.* 2011).

Interestingly, *in vitro* studies also suggest that WRAD, which lacks SET domain, can methylate histone 3 peptide (Cosgrove and Patel 2010; Patel *et al.* 2009, 2011). WRAD can mono-methylate histone 3 peptide by itself but requires the presence of MLL SET domain (even when catalytically inactive) for nucleosome mono-methylation (Patel *et al.* 2009, 2011; Dharmarajan *et al.* 2012).

Two independent studies showed that WDR5 subunit bridges the interaction between MLL and the WRAD complex. WDR5 engages in direct interactions with the conserved arginine-containing WDR5 interacting (Win) motif localized in the C-terminus of MLL protein (Patel *et al.* 2008a, 2008b; Dharmarajan *et al.* 2012). This motif is conserved in SET1 family members and shares sequence homology with histone H3 N-terminus. Correspondingly, Phe133 and Phe263 amino acid residues of WDR5 sandwich the guanidinium of central arginine of the Win motif of MLL (Arg3765). Both *in vitro* and *in vivo* studies suggest that point mutation of either Phe133 or Phe263 in WDR5 or Arg3765 in MLL nearly abolishes the interaction between MLL and WRA subcomplex (Patel *et al.* 2008a; Song and Kingston 2008). However, more recently, it has been suggested that RbBP5-ASH2L heterodimer can bind to MLL directly albeit with weak affinity (Shinsky *et al.* 2014; Li *et al.* 2016).

In addition to the catalytic activity, the components of the WRAD complex are also implicated in the maintenance of the global levels of H3K4 methylation and, stability, product specificity and recruitment of SET1 complexes on the chromatin (Briggs *et al.* 2001; Nagy *et al.* 2002; Dou *et al.* 2006; Steward *et al.* 2006; Smith *et al.* 2011; Ernst and Vakoc 2012; Zhang *et al.* 2015).

3. Non-conventional functions of WRAD

Apart from participation of WRAD in the transcriptional activation of the genes by histone methylation, recent findings suggest transcriptional-independent roles of WRAD in the regulation of the cell cycle progression (figure 1). WRAD along with MLL has recently been shown to regulate the mitotic progression in a transcriptional independent manner (Ali *et al.* 2014). Loss of MLL by RNA interference (RNAi) gave rise to defects in S-phase and M-phase progression. While, after extensive mutational analysis, the regulation of the S-phase progression could be attributed to the activity of Transcriptional activation domain (TAD) of MLL (which acts independently of the SET domain), the domain responsible for influencing mitotic progression was not obvious.

Out of the several mitotic defects observed upon knockdown of MLL, two were quantified: (i) binuclei, arising from defective cytokinesis and (ii) micronuclei, marker of error-prone mitotic segregation (Ali *et al.* 2014). These mitotic defects were phenocopied when each subunit of WRAD was knocked down using RNAi individually, suggesting that the whole MLL 'core complex' was required. Further, we could demonstrate that the WDR5-MLL interaction was crucial for this function of MLL, as a recombinant MLL protein with point mutation in the Win motif was unable to rescue mitotic defects, observed upon the knockdown of endogenous MLL protein. However, further analysis highlighted differences from conventional MLL-WRAD activity, most notable being the non-requirement of MLL SET domain for this function. These observations led us to speculate if WRAD complex also enhances the transcriptional activity of MLL (in addition to its histone methyltransferase activity) using its transcriptional activation domain. We put this hypothesis to test by assessing the Win-motif mutant of MLL for its cell proliferative (S-phase) functions, which is dependent on the TAD domain. Even though, we observed that the mutation in Win motif had no effect on the cell proliferation functions of MLL, later experiments revealed that MLL may not require WRAD for its cell proliferation functions. Hence, we cannot say with certainty if WRAD will affect the TAD activity of MLL or not. But what is clear from our studies is that just the MLL-WRAD interaction is enough for mitotic progression, and neither the transcriptional activity (via the TAD) nor the methyltransferase activity (via the SET domain) of MLL plays any role here (Ali *et al.* 2014). Hence WRAD has a novel function with MLL.

Although this study highlights the emerging non-transcriptional, SET domain- and TAD-independent functions of MLL-WRAD, the underlying molecular mechanism is yet to be investigated. It is likely that WRAD may expand the interactome of MLL, fetching in new specific interaction partners for the proper regulation of mitosis. Alternatively,

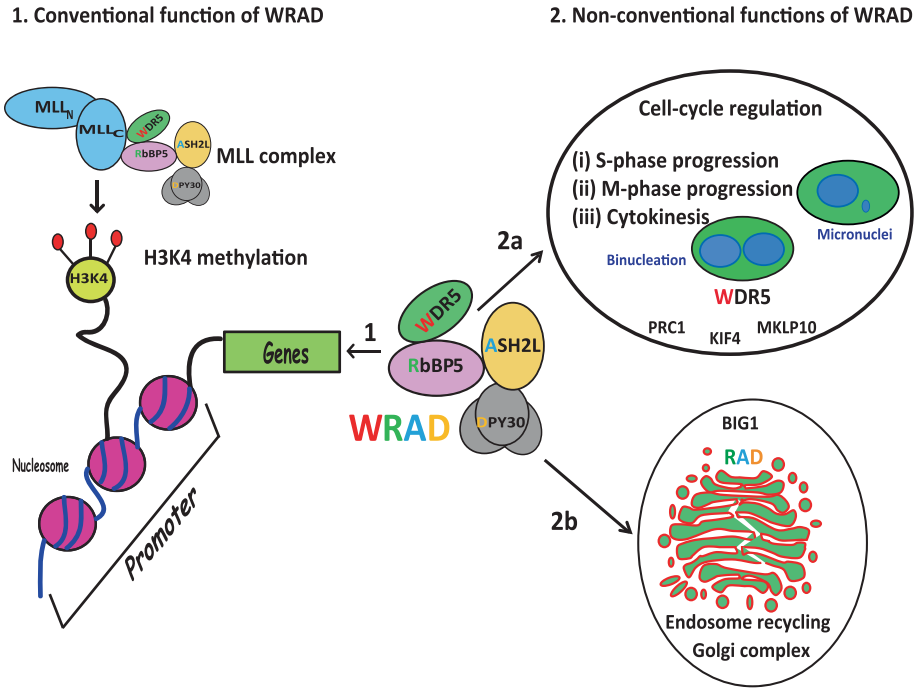

Figure 1. Diverse functional roles of WRAD. The figure shows the model of WDR5, RbBP5, ASH2L, and DPY30 (WRAD) components participating in conventional (**1**) and non-conventional (**2**) functions. (**1**) The WRAD complex associates with MLL and methylates the promoters to regulate the transcription of genes during development. (**2a-i, ii**) Members of WRAD complex participate in cell-cycle regulation controlling S- and M-phase progression. (**2a-iii**) WDR5 subunit of WRAD associates with diverse interaction partners such as KIF4, MKLP10, and PRC1 at the midbody to regulate Cytokinesis; (**2b**) DPY30 component of WRAD interacts with BIG1 to modulate endosomal transport. WDR5, W: Red; RbBP5, R: Green; ASH2L, A: Blue, and DPY30, D: Orange.

it may act as an HMT by itself, performing methylation of non-histone proteins required in mitosis. In any case, it will be exciting to find out the sub-cellular localizations, interactions, and most importantly, functions of MLL-WRAD complex during mitotic progression to discern the underlying mechanism of mitotic regulation by this complex.

Recently, another study demonstrated that WDR5 localizes to the midbody and interacts with the midbody-localized proteins such kinesins (KIF4, and MKLP10) and PRC1 proteins, thereby participating in the proper progression of cytokinesis (Bailey *et al.* 2015). Although this finding could show that central arginine-binding cavity of WDR5 is crucial for its midbody localization, yet the proteins involved in the recruitment of WDR5 to the midbody were not explored further (Bailey *et al.* 2015). In addition, DPY-30 subunit of WRAD has been shown to localize to the trans-Golgi compartments and regulate the recycling of the endosomes (Xu *et al.* 2009). The same study demonstrated that the depletion of RAD (RbBP5, ASH2L, and DPY30) perturbed the recycling of the endosomes (Xu *et al.* 2009). Even though these studies have not tested all subunits of WRAD complex; it is very likely that all of them are involved. Supporting this hypothesis, our studies observed cytokinesis defects with all WRAD subunits, even though only WDR5 has been shown to localize to the midbody (Ali *et al.* 2014; Bailey *et al.* 2015). However, whether MLL is involved in all the above mentioned functions of WRAD is difficult to predict. Interestingly, in size exclusion chromatography of mammalian nuclear extracts, WRA complex in addition to eluting with MLL, also elutes as a distinct ~150 kDa complex lacking MLL, indicating that it may exist as an independent entity in the cell (Steward *et al.* 2006). Therefore, there is a possibility that WRAD may function far beyond the previously studied nuclear HMT function.

4. Future directions

An ever-increasing body of literature has correlated the activity of WRAD with the SET1 family for the histone methyltransferase function. While all these functions of WRAD well connect with its propensity to bind to the gene promoters within the nucleus, recently WRAD complex subunits have been suggested to play roles beyond its nuclear functions. It will be interesting to observe if contribution of WRAD supersedes its thoroughly studied partners to regulate diverse cellular functions.

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