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Methyltransferase Ash1, histone methylation and their impact on Polycomb repression

Eshagh Dorafshan Esfahani

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Fakultetsopponent: Professor Dale Dorsett,
Department of Biochemistry and Molecular Biology, Saint Louis University School of Medicine, Saint Louis, USA.

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Author
Eshagh Dorafshan Esfahani

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Abstract
Antagonistic interactions between Polycomb Group (PcG) and Trithorax Group (TrxG) proteins orchestrate the expression of key developmental genes. Distinct maternally loaded repressors establish the silenced state of these genes in cells where they should not be expressed and later PcG proteins sense whether a target gene is inactive and maintain the repression throughout multiple cell divisions. PcG proteins are targeted to genes by DNA elements called Polycomb Response Elements (PREs). The proteins form two major classes of complexes, namely Polycomb Repressive Complex 1 (PRC1) and Polycomb Repressive Complex 2 (PRC2). Mechanistic details of Polycomb repression are not fully understood, however, tri-methylation of Lysine 27 of histone H3 (H3K27me3) is essential for this process. Using Drosophila cell lines deficient for either PRC1 or PRC2, I investigated the role of H3K27 methylation and the interdependence of PRC1 complexes for their recruitment to PREs. My results indicate that recruitment of PcG complexes to PREs proceed via multiple pathways and that H3K27 methylation is not needed for their targeting. However, the methylation is required to stabilize interactions of PRE-anchored PcG complexes with surrounding chromatin.
TrxG proteins prevent erroneous repression of Polycomb target genes where these genes need to be expressed. Ash1 is a TrxG protein which binds Polycomb target genes when they are transcriptionally active. It contains a SET domain which methylates Lysine 36 of histone H3 (H3K36). *In vitro*, histone H3 methylated at K36 is a poor substrate for H3K27 methylation by PRC2. This prompted a model where Ash1 counteracts Polycomb repression through H3K36 methylation. However, this model was never tested in vivo and does not consider several experimental observations. First, in the ash1 mutant flies the bulk H3K36me2/H3K36me3 levels remain unchanged. Second, in Drosophila, there are two other H3K36-specific histone methyltransferases, NSD and Set2, which should be capable to inhibit PRC2. Third, Ash1 contains multiple evolutionary conserved domains whose roles have not been investigated. Therefore, I asked whether H3K36 methylation is critical for Ash1 to counteract Polycomb repression *in vivo* and whether NSD and Set2 proteins contribute to this process. I used flies lacking endogenous histone genes and complemented them with transgenic histone genes where Lysine 36 is replaced by Arginine. In these animals, I assayed erroneous repression of HOX genes as a readout for erroneous Polycomb repression. I used the same readout in the NSD or Set2 mutant flies. I also asked if other conserved domains of Ash1 are essential for its function. In addition to SET and domain, Ash1 contains three AT hook motifs as well as BAH and PHD domains. I genetically complemented *ash1* loss of function animals with transgenic Ash1 variants, in each, one domain of Ash1 is deleted. I found that Ash1 is the only H3K36-specific histone methyltransferase which counteracts Polycomb repression in *Drosophila*. My findings suggest that the model, where Ash1 counteracts PcG repression by inhibiting PRC2 via methylation of H3K36, has to be revised. I also showed that, *in vivo*, Ash1 acts as a multimer and requires SET, BAH and PHD domains to counteract Polycomb repression.
This work led to two main conclusions. First, trimethylation of H3K27 is not essential for targeting PcG proteins to PREs but acts afterwards to stabilize their interaction with the chromatin of the neighboring genes. Second, while SET domain is essential for Ash1 to oppose Polycomb repression, methylation of H3K36 does not play a central role in the process.

Keywords
Trithorax Group proteins, Polycomb Group proteins, PRC1, PRC2, PRE, histone methylation, histone ubiquitylation, Ash1, SET domain, H3K36, H3K27, *Drosophila*

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