

## DNA damage-binding protein 2 (DDB2) plays a key role in DNA methylation dynamics

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Genomic methylation patterns are the dynamic outcome of counteracting DNA methylation and demethylation mechanisms. In plants, active DNA demethylation is initiated by members of the ROS1/DME family of 5-methylcytosine DNA glycosylases and proceeds through a base excision repair mechanism. However, it is still unclear how DNA demethylation is regulated to maintain and/or reestablish DNA methylation patterns. In this work we describe a direct interplay between the DNA repair factor DNA damage-binding protein 2 (DDB2) and the ROS1-mediated active DNA demethylation pathway in *Arabidopsis thaliana*. We show that DDB2 forms a complex with ROS1 and AGO4 and that they act at the ROS1 locus to modulate levels of DNA methylation and therefore ROS1 expression. We also found that DDB2 represses the enzymatic activity of ROS1. DNA demethylation intermediates generated by ROS1 are processed by the DNA 3'-phosphatase ZDP and the apurinic/aprimidinic endonuclease APE1L, and DDB2 interacts with both enzymes and stimulates their activities. Taken together, our results indicate that DDB2 acts as a critical regulator of ROS1-mediated active DNA demethylation.

