

The impact of post-translational modifications on the activity of ARGONAUTE1 in Arabidopsis thaliana

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Regulation of ARGONAUTE 1 activity

by Post-Translational Modifications in Arabidopsis thaliana



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ARGONAUTE1 (AGO1) is the main effector of post-transcriptional gene silencing in plants, and plays roles in plant development and plant resistance against pathogenes, so, its activity needs to be tightly regulated (Mallory and Vaucheret, 2010; Baumberger and Baulcombe, 2005). It is known that AGO1 is regulated post-transcriptionaly by a feedback loop involving miR168, but less is known about its regulation at post-translational level (Rhoades et al., 2002; Vaucheret et al., 2004). Among the post-translational regulation mechanisms, post-translational modifications (PTMs) allow to quickly modulate protein activity in response to environmental stimuli. In animals, the members of AGO/PIWI family expressed in germline cells have been shown to be methylated on Arginine (R-methylated) (Kirino et al., 2009; Nishida et al., 2009). This modification impacts protein stability and/or small RNAs loading, leading to defects in germ cell maintenance or specification (Kirino et al., 2009; Nishida et al., 2009). In this context, we decide to see if this regulation mechanism is also observed in AGO1 protein involved in meristem formation in A.thaliana.





Figure 2. Predicted methylation sites in AGO1 are located in a disordered N-ter region.

Prediction of long intrinsically disordered **(A)** unstructured regions and protein binding motifs in disordered protein regions in AtAGO1 using IUPRED3 ANCHOR2 (https://iupred.elte.hu/). in (B) Prediction of the 3D structure of AGO1 using AlphaFold protein structure database (https:// alphafold.ebi.ac.uk/ prediction; Jumper et al., 2021; Varad et al., 2021).

ZnAGO ZnAGO1b OsAGO1a NdAGO1-2 BdAGO OsAGO1c NpAGO10 PpAGO1-1 PpAGO1-2 CreAGO3 CreAGO2 VcaAGO CreAGO1	MPRRRKTPK Mprrrktpr Mprrrktpk	ELEEASQA Eleesdra Epeegsry	ISS QALVEAQ 'DT	NSAPAEAG	MAFQLDN SQAAI etegsapsars: stegqaaqpgp	Gyyshqalah Myrkkrnqn Papaatptpa Sapglkrtpa Sapayhpspa	HVRKKRTGPG HVRKKRTGP- H-RKKKTEPR IPGGGEGSQPQ ISASGPSGSAA ILPSGTSA	GSGETSGES -GESSGET AGESSGTQQA EGSGGSDQSSQ FAPPPTATPY- FSPGPYALPYP FSPASDRTSYP	SGASGQGSSQ SGAPGQGSSQ TGAPGRGPSQ RSSPAPAPAPAP VQEPAPQDI YQNADPQPAE PAAYSPAPSP.	QPERT-QQF RPQATQQGA RPERA-QQH APQQYYEGG RDEATSQGR RQGHGQAHS IQHPSP	YGGGR IRGGGQHQVR IGGGGHQPAN IRGGGPGGYQ IRAATAGS-Q IRNYGEGG-Q YRRIGGGY-Q	GHYPQQG GgYPGHGYPP PQYAQQA GgGGGGGPGGY GyyPP GQCSYQPQGQ GQGP	GHGGGGH SEHPGGGPP GRGGGGGPG MGSRRPRL MASRRPT- GTQSG-GQG GQESGQGQG GQAFAQGQG MSGRGRGGG- MSGRGRGGG- MSGRGRGGAS	QGRORHYQG EYQPRGYQG GYQGGGGGYQG PGFGDDGGG HRHHTEAPD RGRGRPRPD SGQGRDESS QGSNEYS GGGYGGGGG GGGYGGGGG GGGYGGGGG
onsensus	•••••	•••••	••		•••••	••••••	.nt	•••••	•••••	••••••	•••••	g	••• <mark>פרפ</mark> -פ	•8•88•888
	131 14	0	150	160	170	180	190	200	210	220	230	240	250	260
SIAGO PotriAGO MdAGO1-1 GmAGO1-1 GmAGO1-2 VvAGO BdAGO1b OsAGO1b ZmAGO1c ZmAGO1c ZmAGO1c ZmAGO1a MdAGO1-2 BdAGO OsAGO1c MpAGO10 PpAGO1-1 PpAGO1-2 CreAGO3 CreAGO2	RGGGGGAP RGGRGVPQQ RS-RGMP RS-RGMP RGGRGPQ PGGPA RGGQGSHHP RGGPGPHHL RGVP-PLLP RGGPGPHHL RGVP-PLLP RGGPGPHHL RGGQGRG GKLRGGDPD SGGPPGRGR SDVSLGRGR YGGGGGGGR	RGGIAP RGGHQ- QQYGGPQE RGGHAP RGGHAP GGGPPE GGGPPE GGGPVE AQQGPQ RGRAA RSWPGHSG GMCGGERG GMCGGDRG GGGGGS		GPP GPP GPQ GPQ GPQ GPQ GPQ GPQ GPGGGGGGGGGG	EYQQRGRGG EYQQRGRGG EYQQRGRGGG EYQGRGRGGG EYQGRGRGGG PPEYQQRDYQG PPDYQGRGGSG EYQGRGRGGSG EYQGHGGEYI YQGRGGPP GHPPQQQYGYG SYHYNGQGAAPI YQGRGGPP GHPPQQQYGYG SYHYNGQGAAPI QQQQQGRGC PHSTLHQNYPG PLNAPYYNAPG RGGGGGGGGGYGG GGGRGGGGGGYGG	RGHPGGGP-P SHHPGGGP-P SHHPGGGP-P QGRGG		GPQRRGGIGG GSYGGGRGGGG GSYGGGRSGGG GGYGGGRSGGG GGYGGGRSGGG GGYGSGRSGGG AAAAYESGSRS GGPRPRGG-MPI GGPRPRGG-MPI GGARSRGG-IS GGGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GG-GGRSRGG-MPI GGG-GGRSRGG-MPI GGG-GGRSRGG-GG GGG-GGG-GGGG-GGGGG-GGGGG-GGGGG-GGGGGG	RGA RGG RGG GGMGSGRGV GGGMGSGRGV QPYGGHRGG QPYGGHRGG QPYGGHRGG QPYGGHRGG QPYRGGHVGG QPYRGGHVGG QPYREVH TTVTVAS HGGVPFQAAH HYGRPSR PPHYVRPSTR GGYGGGGGGGG GGGYGGGGGGGG	PSGGS PSSGG- PPSPGGP GPSYGGP GPSYGGP GPSYGGP GGRSVPSG SGGRSVPSG SGGRSVPSG SVGRNVPFG SVGRNVPFG SVGPIVPSG SGGHGVPFS SGGHGVPFS SSSTTATFS PGGPSFGGC SSSTTATFS PGGPGFPLFF APQQTHPLF GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	SRPYPELH SRPPAPELH TRPQFPELH SRPPAPELH SRPPAPELH SRPAPELH SRPTYPELH SRTYPELH SSRTYPELH SSRTYPELH SSRTYPELH SRTYPELH SRTYPELH SSPLAPELR ADQYPQLGR HRPLPQGGR HRPHQPAGR SYYSPQQAAA	ATTESTITAN- QATETPHQPY QATPAPYPAGA QATSVQFYQT QATSVQFYQT QATSVQSYQT QATSVQSYQT QATQASYAA- QAPYVQYQAP QAPYVQYQAP QAPYVQYQAP QAPYVQYQAP QAPYVQYQAP QAPYVQYQAP QAPYVQYQAP QASQDQYQAT QATSAPYYA- QATYDDAPHE QAIME-APR- APAPYPSSH- GPSYLGPSHG SLDAILKSAK TLDYYLKSAR	PYG G G 	SSQF ILSE RPAETYSEA TTOPTPSEA TSPPYYYGS SSQPALSEA SSQPASSEA TPHRVPSEA IS-PSASGA IS-PSPSGP VS-PSPSGP VS-PSPSGP VA-PSPSRT TPQATGPAP ISSPEAAGS PSELAQ GTQPSSATA SASPPSYGP ASPSSSYGP APVKVETTA
CreAG01	00.0	000	110	H	LSKYQLPRRAG	Ransatpysa	RYSDSANRPG	GRGGGGQPGRGG	GGRGGGSGRG	SSSSTAPK		SFATRLFTGL	PLGP¥EPRQ	LANGHAARP
onachada	• 55 • 5 • • • •	**555***	•••••	5 ••••••	+++342+2, 88		••••• <mark>68</mark> 1	55·····5666	••••88••88	•••	bb beru	4aa.	•••••	••• <mark>*</mark> ••••

Alignment of GRG/RGG containing regions of homologs of AtAGO1 from ten different plant species (At: Arabidopsis thaliana, Cre: Chlamydomonas reinharditii, Vca: Volvox carteri, Mp: Marchantia polymorpha, Pp: Physcomitrium patens, Bd: Brachypodium distachyon, Os: Oryza sativa, Zm: Zea mays, SI: Solanum lycopersicum, Vv: Vitis vinifera, Potri: Populus trichocarpa, Md: Malus *domestica*, Gm: *Glycine max*). Highlighted arginines by red asteriks correspond to conserved arginines in RGG/ GRG motifs (conserved in 40% of the sequences).

AGO1 is PRMT5-dependent. AGO1 IPs performed on total protein extracts from Col0 The prmt5-4 buds. and Immunoprecipitated AGO1 protein, sDMA and aDMA modifications are detected US

and the sDMA deposition on Immunodetection of AGO1 on total protein extracts of closed buds and opened flowers from Col0 *prmt5-1*. The and membrane with stained is colloidal coomassie as a loading control.

sing specific antibodies.	col	prmt5-2	prmt5-1
Normalization factor	1.30	1.38	1.00
Spectra/AGO1	11757	1660	2287
SC% AGO1	66%	68%	68%
Mascot threshold (p>0.05)	32	32	33

	score max	#spc	score max	#spc	score max	#spc
R30(diMe)	39	9	41	10	37	5
R34(diMe)	23	2	61	11	53	22
R59(diMe)			29	3	26	8
R62(diMe)			97	13	93	20
R83(diMe)			28	2	21	2
R85(diMe)			28	2	27	4
R94(diMe)			18	1	24	2
R101(diMe)	34	10	46	11	45	15

Figure 5. sDMA sites are converted in aDMA sites in *prmt5* mutants.

Identification of dimethyl-R sites by MS/MS analysis on AGO1-IPs obtained from Col0, prmt5-2 & prmt5-1 closed buds extracts.

4. The impacts of the *prmt5* mutation on mRNA and miRNA levels



Figure 7. Differentially detected transcripts are mainly upregulated in *prmt5* mutants.

Venn diagrams obtained from polyA-RNAseq analyses, performed from Col0, prmt5-2 and prmt5-4 closed buds total RNA. Downregulated (left) and up-regulated genes (right) in prmt5 mutants buds compared to Col0. The number of genes expressed in both *prmt5* mutants and that do not differ from Col0 are indicated at the bottom right of each panel.



5. The localization of AGO1 in *prmt5*



Figure 9. GFP-AGO1 subcellular localization is not affected by *prmt5* mutation.

Confocal images obtained from root tips of 5-days-old GFP-AGO1/ ago1-3; GFP-AGO1/ago1-3/prmt5-1, transgenic seedlings. From left to right: GFP signal, transmission acquisition and merged image.

Figure 8. Only few miRNAs are differentially loaded into AGO1 in prmt5 mutants..

(A) Mean expression levels of individual miRNAs in closed buds and open flowers of prmt5-2 and prmt5-4 plants relative to Col-0 plants. Horizontal segments indicate the median of the expression levels. Each dot corresponds to a single miRNA. Dark and light dots show differentially (false discovery rate adjusted P-value, 0.05) and not differentially accumulated miRNAs. respectively. (B) Bar plot representing the variations of miRNAs loading into AGO1 (down-regulated, unaffected and upregulated) in prmt5-2 and prmt5-4 compared to Col0. AGO1-IP were performed on closed buds extracts.

Conclusion

AGO1 protein is symmetrically and asymmetrically dimethylated in closed buds in A.thaliana, and the sDMA deposition is PRMT5-dependent. Methylation sites are conservationally organised as a cluster in N-ter region of AGO1 homologs among plants. The absence of sDMA on AGO1 in *prmt5* may be compensated by the presence of aDMA on the same sites. So it will be crucial to get rid of methylation marks to study methylation effects on AGO1. We will perform further investigations using AGO1 non methylable forms.