Supporting Information

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SI Materials and Methods

Plant Materials. *nrpe5a-1* (FLAG 607D12), *nrpe5b-1* (SALK_134107) *and nrpe5c-1* (GABL454F05) homozygous plants were genotyped by PCR (1, 2, 3). The endogenous copy of the gene was detected using primers 149/152, 513/514 and 515/516 respectively. The T-DNA insertions were followed using primers Tag6/149, LBA1/513 and 8409/515.

Semiquantitative and Real-Time PCR Amplifications. Primers 786/787 for EF1–4 α were used as calibration control and primers 603/ 604, 149/152, 513/514 and 515/516 were used for *NRPB5a*, *NRPE5a*, *NRPE5b* and *NRPE5c* respectively. Primers LTR625-F and LTR625-R for *IG/LINE* and GAPA-F, GAPA-R for *GAPA* (At3g26650) a constitutively expressed gene as control, were reported in Huettel *et al.* (4). RT-PCR conditions were as follows: 94°C for 3 min, followed by 22 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, for GAPA or 28 for IG/LINE. Real-time PCR amplification was performed using the same primers and LightCycler 480 SYBR Green I Master (ROCHE Diagnostics) to analyze expression of IG/LINE. Data were analyzed using Light cycler software (Roche). Relative transcript levels were calculated using Δ CT method (5). GAPA was used as internal reference.

Immunoprecipitation. For PolII immunoprecipitation, plant extracts were prepared as described in Li *et al.* (6). Dynabeads M-280 Sheep anti-Mouse IgG (Invitrogen) were used to bind the anti-NRPB1 8WG16 antibody (Abcam) using 3 μ l for 100 μ l of beads resuspended in PBS/BSA (5 mg/ml BSA in isotonic (PBS) pH 7.2–7.6 as recommended by the manufacturer) and rotated O/N at 4°C. After 3 washes in isotonic PBS, the coated Dynabeads were resuspended into the plant extracts and rotated for 2h. After 3 washes in IP buffer, the bound material was recovered by a direct addition of SDS/PAGE loading buffer. The same experiment was carried out without any antibody as a negative control.

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- Samson F, et al. (2002) FLAGdb/FST: a database of mapped flanking insertion sites (FSTs) of Arabidopsis T-DNA transformants. Nucleic Acids Res 30:94–97.
- Rosso MG, et al. (2003) An Arabidopsis thaliana T-DNA mutagenized population (GABI-Kat) for flanking sequence tag-based reverse genetics. *Plant Mol Biol* 53:247–259.
- 4. Huettel B, et al. (2006) Endogenous targets of RNA-directed DNA methylation and PolIV in Arabidopsis. EMBO J 25:2828–2836.
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25:402–408.
- Li CF, et al. (2006) An ARGONAUTE4-containing nuclear processing center colocalized with Cajal bodies in Arabidopsis thaliana. Cell 126:93–106.

A+DDDE-		MI MERSI VOI VOI OVELNOM DODCARTAD
ACRPBSA	1	MLTEEMLKRIJIRIQKALAOMIRDRGHFTADS
AtRPB5b	1	MSDMDDBITRIPKVRRVEQMIRDREYTIEES
OsRPB5	1	INSAGLVTEEVMVGRLVRIRRTVMOMLRDRGYLVVE
ScRPB5	1	MOOENERN I SRIWRAPPROVED BY TOP
Habbbc		NDDEREMYDIWY IDYN IVOL CHDDONI UMOL
II SKPD5	-	
DmRPB5	1	MDDEADTYKLWRIRKHIMOESHDRCHUUTOI
AtRPD5a	1	MEVKGKETASVLCLSKYVDLSSEDSHRYYLARRNGLOMLRDRGYEVSDH
At RPD5b	1	MEGKGKETVVGHSTSKSSVICHKYVLARETTMEMORDRGYDVSD
Atppbsa	î	MPPTWAPPCCCPNVPSTPDDCTNCTSKTPDTCCTBSKDPVLADTAAPPWIDDDCAVPVNP
ACREDSC	-	ALLIAALLOCCLAVESII DOGIACISKILDIGGIBSKAI I LAATIAT LAAKAAA
OSRPD5	1	MAAEMEVDDVDVHEVPECIASMIDRGSVESH <u>RHF</u> LA <u>RRW</u> AME <u>MIRDRGM</u> SVPEA
SaRpoH	1	
PfRpoH	1	
AtRPB5a	32	DITMT KQQEIRKHCONMKREDUVT-KAKRNONSDQLYIFEPDEAKVGVKTI
AtRPB5b	33	DUNLKREEFVORFCKTMNKVNKEAUFVSANKGPNDADKIYVFYPEGPKVGVPV
Os RPB5	37	PROAMGRR DELEKYGES FHREDIL, TNKYKKNDPSDOTYVPPPNDDKVGMCH
Conne	2.4	
SCRPDS	34	IVELPTEDT KARICDSAGRPORKAMSTOANPTEESISKTPDAGSENVELCDEPSVGVAT
HSRPB5	32	DIDOTTEEEKAQPEDKESEGRPRRTDITVLVAHNDDPTDQMFVPEPEEPKVGIRT
DmRPB5	32	HDQTLEQEKEMFGDKPSEKRPARSDIIVLVAHNDDPTDQMFVFPEEPKIGIKT
At RPD5a	50	DINLSIH DERTVYCERD DVDRIGT SALHRSDSTKKVKTVPPGTSMVKVNA
Atppnch	46	DINISIO
ACREDOD	40	
AtRPD5c	61	BISLITTSEIRSVPGEREELERURICVPLRSDPKKKILVVPMGTEPITVKS
OsRPD5	55	DIARTUPERAWWAEKPGIERDAFTTTLVSDPSKKVOLVPCPPEPVKIAT
SaRpoH	1	
DfDnoH	÷.	
FIRPOR	1	
AtRPB5a	82	K-MYTNRMKSENVPRALLVVOONLEPPARTCISEISSKFHLEVFOEAEMIVNIKEHVIV
Atppssh	87	IN EVATEMPODEVED CTVVVPMATUA PARMAVSELNEMI, TEVEPEA PAVUNTUPHENAV
ACRIDOD		
OSRPB5	88	K-KIVEMMKABNVSRAVLVLOONLUPPARSFLOELEPKIHLBIFOBAELFINIKEHVLVF
ScRPB5	94	<u>H</u> -TFVIHIQEKMFQTGUFWYONNIWPSMMKLVPSIP-PATIETENEAALVVNUUHHEUV
HsRPB5	88	K-VYCORMOE IITRALIVVOOGMTPSAKOSLVDMAPKYILEOFLEOELLINITEHELV
Dmppp5	88	U-TYCTRMOEENT HRATIVVVOGGMUPSAKOSLVDMARKYTLEOPLESETATINT TEHETAV
BADDDE-	101	
ACRPDSa	101	RSVVADIESQUITITGELEVEONNVINOALKAIEEFSFRVININOITDEGVNIVKIISEK
AtRPD5b	97	RVIAADVLSRIMITGLULVLOSHIMNOMLKAVELPSPKVBLEEITDLOOVNVSKHVIR
AtRPD5c	112	RALHIOISNNVGLHAMILVLOSKMNHPAOKALTTPPFTVETPPIEDIDVNLTKHIOO
Os PPD5	106	RETYLOTKEENLSPLULTLOSKILSPAREATKEIPKPKVDIEOATDIAVNITKEVIK
Callacy	12	
Sakpon	13	
PfRpoH	7	FSUPDavio
A+DDB5a	141	PHOWING PERKING PERKING TO THE TOWARD THE PROTOCOVER THE PROPERTY OF THE PROTOCOVER T
ACREDJa	141	
ACRPB5D	147	NI IVEDDQARKKEETATYTVQDTQEPRILVTDPFARYYGEKRGQVVKIRRSDATSLDYYT
OsRPB5	147	<u>EHQVLNNEEKKTLLERYTLKETQLPRIQITDPIARYYGLRRGQVVKIIRPSETAGRYVT</u>
ScRPB5	152	KHIRLSSDEKRELLKRYRLKESOLPRIQRADPVALYLGLKRGEVVKIIRKSETSGRYAS
HsRPB5	147	ERVYMTKEEVSELLARYKIRENOLPRIOAGDPWARYEGIERGOVYKII PPSETAGPYITT
DmDDBE	147	PHUMMMURPHOPHICS BY KING NMIMPTON CORPUSED VECTOR COMPLETING COMPLETING
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AtkPD5a	159	OHOVENDERSTTTEEKKPSTEEKOIPRISKKDAIVRYYELEKGOVVKVNYRGELTESHVAI
AtRPD5b	155	KHQVINDKEKESIIKKFSIEEKQIPRLSSKDPIVRYYGIETGOVMKVTYKDELSESHVT
At RPD5c	170	KI ETUNKEEKEODORKHALEDKOLPYLOEKDSPYRYYGIKKKOVYKTTYSKEPVCDEVT
ORPDDE	164	KIPWISA DOGA KIA KEPUNUPD SOLDENI, PTIDA WADVY OPDIKOTVA PUTUD COL TO PUAL
OSRF05	104	A DE LA VILLE A LA DE OUTER DE OUT
закрон	23	KHEILQLMAAYKELGIKPEOLWIRASDEVAKSICAKPEDIIKUTRKSPPTCESVT
PfRpoH	17	EHRVLSEEPKKALLEKYKITLAOLPOIKASDPAVKALGAKPGDVIEIKRKSPTAGVYYY
-		
A+DDDC-		
ACRYBBA	201	
AtRPB5b	207	BPAV Torr
OsRPB5	207	RXWV Jaw
ScRPB5	212	BICM
Hepppe	207	
ASKPD5	207	Assembly
DmRPB5	207	REVC ASSENDTY
AtRPD5a	219	
AtRPD5b	215	
Atppbsc	220	
OcBBDE	224	
USKPUS	224	
SaRpoH	83	RAMIAC
PfRpoH	77	

Fig. S1. Amino acid sequence alignments of Arabidopsis thaliana RPB5-type proteins with their counterparts in animal, fungi and archaeal RNA polymerases. Boundaries of both the eukaryote-specific N-terminal Jaw domain and the conserved C-terminal Assembly domain are indicated gray/dark lines. Abbreviations: Sc, Saccharomyces cerevisiae; At, Arabidopsis thaliana; Os, Oryza sativa; Hs, Homo sapiens; Dm, Drosophila melanogaster; Pf, Pyrococcus furiosus; Sa, Sulfolobus acidocaldarius

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Fig. S2. Evolutionary relationships between RPB6-, 8-, 10- and 12-type proteins and related factors (*A*, *B*, *C*, and *D* respectively). The unrooted phylogenetic trees were inferred from the full-length protein alignment. Abbreviations: Sc, Saccharomyces cerevisiae; At, Arabidopsis thaliana; Os, Oryza sativa; Hs, Homo sapiens; Dm, Drosophila melanogaster; Pf, Pyrococcus furiosus; Sa, Sulfolobus acidocaldarius.

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Fig. S3. NRPE5 genes expression in wild-type plants and homozygous mutants shown by RT-PCR on flower total RNA. gDNA represents the genomic DNA control. $EF1-4\alpha$ is used as a control to standardize the RT-PCR reactions.

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Fig. 54. Immunoprecipitation of PollI. PollI complex was immunoprecipitated from Columbia inflorescences using the anti-NRPB1 8WG16 antibody (+). As a control, the same experiment was performed without antibody (-). The "input" lane corresponds to the plant extracts prior immunoprecipitation. These samples were used run onto SDS/PAGE gels and subjected to Western blots using anti-NRPB1, anti-NRPB5a and anti-NRPE5a antibodies.



Fig. S5. Complementation of the *nrpd1a-4* mutant. (*A*) *AtSN1* DNA methylation in *nrpd1a-4* is rescued by transgenes expressing a Flag-tagged version of NRPD1a. Two independent T1 transgenic lines are shown. Undi represents the Col-O undigested DNA control. (*B*) 55 DNA methylation in *nrpd1a-4* is restored by a transgene expressing a Flag-tagged version of NRPD1(NRPD1a). Genomic DNA from wild-type, *nrpd1-4* and two independent T1 transgenic lines were digested by HaellI and subjected to Southern Blot analysis using 55 probe.

HaeIII

DN A S



Fig. S6. sRNA accumulation in *nrpd1/nrpe1* mutants at PolIV(PolIVa)/PolV(PolIVb)-dependent loci. Analysis of sRNA accumulation by Northern blot done with 30 µg of total RNA from inflorescences from wild-type (Col-0), *nrpd1–4* and *nrpe1–11*.

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Fig. S7. Loss of CNN methylation at 5S and AtSN1 loci in wild-type and nrpe1 mutants. (A) Haell digested DNA were used as templates for AtSN1 PCR reactions. Undi represents the Col-0 undigested genomic DNA. (B) Southern blot analysis of methylation at the 5S locus. Genomic DNA from wild-type, nrpe1–11 and nrpe1–3 mutants were digested by HaellI.

Table S1. Primers and peptides used in this study

Name

Pe

Primers used for genotyping and RT-PCR
149
152
352
462
463
465
513
514
515
516
603
604
786
787
Primers used for sRNA probes
5S
45S
si02
Rep2
SimpleHat
TR2558
TR2558
miR159
U6
Peptide used for antibody production

NRPE5a_ pep: TKHSLKPQHQVLNDE

NRPE5c_ pep: KEPVGDFVTYRCII

Sequence

5"-gtgaaagggaaagagacagct-3" 5"-tcaccacacatcggaaggc-3" 5"-aagcttctttcgtttagagatcatcttaaggcggcg-3" 5"-gtcgacatggaagacgattgtgaggagcttcag-3" 5"-ccatgggcgggttttcggagaaaccaccgga-3" 5"-ggatccgtcgacccatcagtaaggaaaatttagatacg-3" 5"-gggaaagagatagtggttgg-3" 5''-agaaacgcatcggtagtaac-3'' 5''-acaatggccgaagaagggtgc-3'' 5"-acaattgtaagcacacaccaaggacac-3" 5"-gatgttgacggaagaggaggtc-3" 5"-cgataggtaacataacgaccagccg-3" 5"-ctaaggatggtcagacccg-3" 5''-cttcaggtatgaagacacc-3'' 5"-atgccaagtttggcctcacggtct-3" 5"-gtctgttggtgccaagagggaaaagggctaat-3" 5"-gttgaccagtccgccagccgat-3" 5"-gcgggacgggtttggcaggacgttacttaat-3" 5"-tgggttacccattttgacaccccta-3" 5"-ttcatcagcatgaccgatagctta-3" 5"-tttgttcaatcattcattggccat-3" 5"-tagagctcccttcaatccaaa-3" 5-aggggccatgctaatcttctc-3