Supporting Information

Tsuchiya and Eulgem 10.1073/pnas.1312545110

SI Text

Sequence Analyses of $RPP7_{Krazo-2}$ and $RPP7_{Koch-1}$. Resistance to *Peronospora parasitica* (*RPP7*)-like genes in Krazo-2 and Koch-1 were structurally highly similar. The genomic, cDNA, and deduced amino acid sequences of their *RPP7*-like genes were 98.4%, 96.9%, and 94.5% identical, respectively. In addition, the sequences of the *RPP7*-like genes in Krazo-2 and Koch-1 showed high similarity to that of respective genomic locations of Col-0 *RPP7* (Fig. S5*B*). Moreover, the genomic region from intron1 to exon2 of *ECL*, which is unique to *RPP7* in the Col-0 genome, is also conserved in the *RPP7*-like genes (Fig. S5*B*). Finally, blast searches of the deduced protein sequences of the *RPP7*-like genes against the TAIR10 CDS database (www.arabidopsis.org/Blast/index.jsp; TBLASTN option) gave the highest identity and the smallest E value for Col-0 RPP7 (identity = 88%, E = 0.0 for the gene in Krazo-2; identity = 91%, E = 0.0 for the gene in Koch-1). Taken together, we concluded that these cloned and sequenced genes in Krazo-2 and Koch-1 are the orthologs of Col-0 *RPP7* and were therefore named $RPP7_{Krazo-2}$ or $RPP7_{Koch-1}$, respectively.

SI Materials and Methods

Transgenic Lines. The transgenic complementation lines expressing N-terminally HA-tagged EDM2 driven by EDM2 native promoter in the *edm2-2* background ($E2_{pro}$:*HA-E2*) were generated as described previously (1). Fourteen independent $E2_{pro}$:*HA-E2* lines were recovered. Among the complementation lines, we selected three homozygous lines that harbor a single copy of the transgene and whose transcript levels of *RPP7* are intermediate and partially ($E2_{pro}$:*HA-EDM2a* and *b*) or almost completely ($E2_{pro}$:*HA-EDM2c*) reverted to those in wild-type Col-0 (Fig. S1*A*). This variation of the extent of complementation in *RPP7* transcript levels is likely due to positional effects of *HA-EDM2* transgenes. To generate silencing lines for $EDM2_{Krazo-2}$, $EDM2_{Koch-1}$, and Col-0 *EDM2* in Krazo-2, Koch-1, and Col-0, respectively, the *EDM2* cDNA sequence conserved in these accessions was PCR-amplified using primers EDM2RNAi-F and EDM2RNAi-R (Table S1). The resulting PCR product was cloned into the getaway entry vector pDONR-zeo (Life Technologies) through BP reaction and sequenced to verify the insert. The cloned silencing trigger was recombined into the pJawohl8-RNAi vector (GenBank accession no. AF408413; kindly provided by Imre E. Somssich, Max Planck Institute for Plant Breeding Research, Cologne, Germany) through LR reaction generating the plasmid pJawohl8-EDM2. This binary vector was introduced into *Agrobacterium tumefaciens* strain GV3101(pMP90RK) and used to transform each *Arabidopsis* accession by the floral dipping method (2). The transformants were identified through BASTA selection.

RNA Isolation and qRT-PCR Analysis. Plants were grown in a controlled culture room at 22 °C under long-day conditions (16 h light/8 h dark) with white light illumination. Total RNA was isolated from 2-wk-old seedlings using TRIzol reagent (Life Technologies). One microgram of total RNA was incubated with 1 unit of DNase I, RNase-free (Thermo Scientific) in a total volume of 10 μL at 37 °C for 30 min. After inactivation of DNase I by adding 1 μL of 50 mM EDTA and heating to 65 °C for 10 min., RNA was reverse-transcribed using 200 units of Maxima reverse transcriptase (Thermo Scientific) and 100 pmol of oligo $(dT)_{18}$ or 20 pmol of gene-specific primers in a sample volume of 20 μL. cDNA aliquots (0.5 μL) were used as template for real-time qPCR using an MyiQ real-time PCR detection system (Bio-Rad). Each 25 μL of reaction mix contained 12.5 μL of iQ SYBR green Supermix (Bio-Rad) and 0.6 μM gene-specific primers. Melt curve analysis was performed to confirm specific PCR amplification. For each reaction, the threshold cycle value (Ct) was determined by setting the threshold within the logarithmic amplification phase. Serial dilutions of total cDNA were used to calculate the amplification efficiency of each primer set according to the equation $E = 10^{[-1/\text{slope}]}$. Relative transcript levels were calculated by a comparative Ct method using *Actin8* as a reference gene (3). To determine the ratio between transcript isoforms, the absolute copy numbers of cDNA molecules were measured by the standard curve method. RNA was reverse-transcribed with oligo$(dT)_{18}$, and the same volume of cDNA aliquot (0.5 μL each) from the same RT reaction was applied for the measurement of each RNA isoform. Templates to generate standard curves were plasmid vectors harboring full-length cDNA of *ECL* (for measuremens of ECL transcripts) or RPP7-coding mRNA (for measurements of total exon1-containing and RPP7-coding transcripts). The primers used for RT-PCRs are listed in Table S1.

Chromatin Immunoprecipitation. Aerial parts of 3-wk-old plants (or 2-wk-old for the time-course experiments of *Hpa*Hiks1 infection) were harvested and fixed in cross-linking buffer [1% formaldehyde, 0.4 M sucrose, 10 mM Tris·HCl (pH 8.0), 1 mM EDTA, 1 mM PMSF] for 15 min at room temperature under vacuuming. Closs-linking was quenched with 0.125 M glycine, and the materials were washed three times with water, dried with towels, frozen, and stored at −80 °C until use. Chromatin was extracted by grinding fixed tissues to powder in liquid N_2, followed by homogenization in nuclei isolation buffer [0.25 M sucrose, 15 mM Pipes (pH 6.8), 5 mM $MgCl_2$, 60 mM KCl, 15 mM NaCl, 1 mM $CaCl_2$, 0.9% Triton X-100, 1 mM PMSF, 1% proteinase inhibitor mixture for plant cell and tissue extracts (Sigma-Aldrich)]. The homogenate was filtered through two layers of miracloth, and the filtrate was centrifuged for 20 min at 11,000 × *g*. The pellet was resuspended in nuclei lysis buffer [50 mM Hepes (pH 7.5), 150 mM NaCl, 10 mM EDTA, 1% SDS, 0.1% sodium deoxycholate, 1% Triton X-100, 1% proteinase inhibitor mixture]. The extracted protein-DNA was sheared by sonication to ∼350- to 800-bp fragments and centrifuged. Supernatants were carefully quantified using a spectrophotometer. A fraction of the sonicated supernatants was saved and, after cross-linking reversion, was used as input control in qPCR measurement. For chromatin immunoprecipitation, 10 μg of chromatin diluted ten times with ChIP dilution buffer [50 mM Hepes (pH7.5), 150 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Triton X-100, 1% proteinase inhibitor mixture] was precleared with 60 μL of Dynabeads Protein A (Life Technologies) for 1 h at 4 °C. The precleared protein–DNA complex was immunoprecipitated overnight at 4 °C with 60 μL of Dynabeads Protein A bound by an appropriate antibody. Also, a no-antibody negative control was performed by omitting antibody addition. After incubation, the magnetic beads were washed twice with low salt wash buffer [20 mM Tris·HCl (pH 8.0), 150 mM NaCl, 1.0% Triton X-100, 2 mM EDTA, 0.1% SDS], twice with high salt wash buffer [20 mM Tris·HCl (pH 8.0), 500 mM NaCl, 1% Triton X-100, 2 mM EDTA, 0.1% SDS], twice with LiCl wash buffer [10 mM Tris·HCl (pH 8.0), 250 mM LiCl, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM EDTA], and twice with TE buffer [10 mM Tris·HCl (pH 8.0), 1 mM EDTA); the precipitated chromatin was eluted twice with elution buffer (0.1 M $NaHCO_3$, 1% SDS). Cross-linking was reversed overnight at 65 °C in the presence of 0.3 M NaCl, and samples were treated with 2 μg of

proteinase K for 3 h at 50 °C. Samples were then purified with the QIAquick PCR Purification columns (QIAGEN), and DNA was eluted twice with 50 μL of buffer EB. Two microliters of eluted DNA was applied for qPCR. Two microliters of ChIPed DNA and 1% inputs were applied for qPCR analysis. ChIP signals were calculated using the $2^{\Delta Ct}$ method as % of precipitated material relative to the amount of the input sample (% input). For H3K9me2 measurements, values of % input were further normalized to H3 occupancy to adjust for nucleosome density when appropriate. Fold enrichment of HA-EDM2 signals in the *E2$_{pro}$:HA-E2c* line was shown relative to nontransgenic Col-0 levels.

1. Tsuchiya T, Eulgem T (2013) Mutations in EDM2 selectively affect silencing states of transposons and induce plant developmental plasticity. *Sci Rep* 3:1701.
2. Clough SJ, Bent AF (1998) Floral dip: A simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* 16(6):735–743.
3. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29(9):e45.

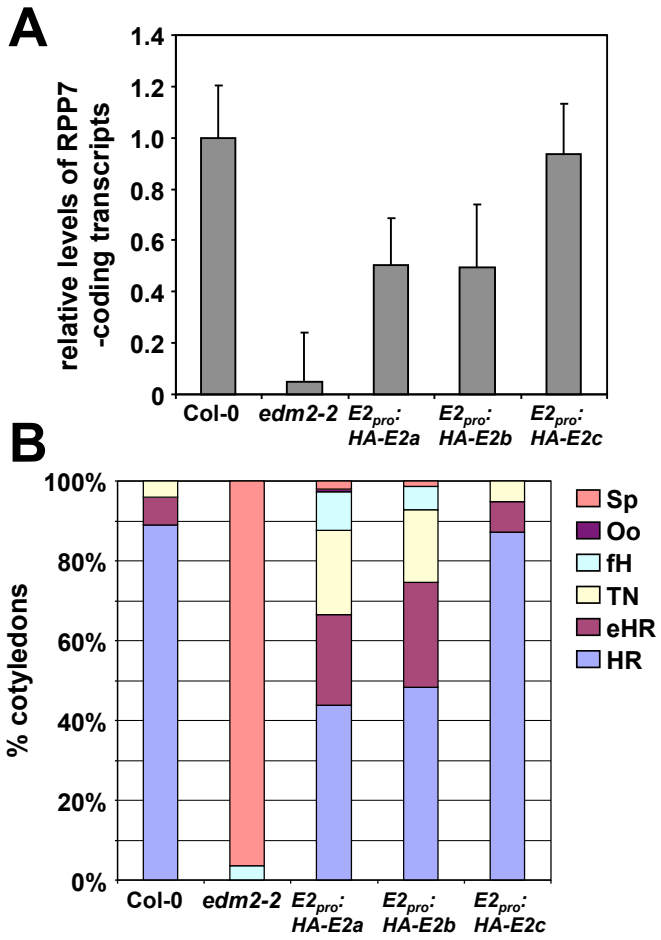

Fig. S1. Levels of RPP7-coding transcripts are correlated with levels of plant immunity. (*A*) *RPP7* transcript levels determined by qRT-PCR. *ACTIN8* (*ACT8*) served as an internal control. *E2$_{pro}$:HA-E2*: *EDM2* complementation lines expressing in the *edm2-2* background HA-tagged EDM2 driven by the native EDM2 promoter (E2$_{pro}$). In *E2$_{pro}$:HA-EDM2a* and *-b*, transcript levels of RPP7 are intermediate and only partially reverted to those in Col-0. In *E2$_{pro}$:HA-EDM2c,* transcript levels of RPP7 are almost completely reverted to those in Col-0. (*B*) Two-week-old *Arabidopsis* seedlings were spray-infected with 5×10^4 *Hyaloperonospora arabidopsidis* Hiks1 spores ml^{-1} and scored for disease symptoms 7 d later. Individual cotyledons of the infected plants were assigned to defined categories based on the strongest type of infection symptom they exhibited using the following classification scale: discrete hypersensitive response (HR) sites < extended HR sites (eHR) < trailing necrosis (TN) < free *H. arabidopsidis* hyphae (fH) < *H. arabidopsidis* oospores (Oo) < *H. arabidopsidis* sporangiophores (Sp). HR is typically associated with full immunity; eHR and TN indicate partially failed immunity; fH, Oo, and Sp are typically observed in fully susceptible plant tissues (1).

1. Tsuchiya T, Eulgem T (2011) EMSY-like genes are required for full RPP7-mediated race-specific immunity and basal defense in Arabidopsis. *Mol Plant Microbe Interact* 24(12):1573–1581.

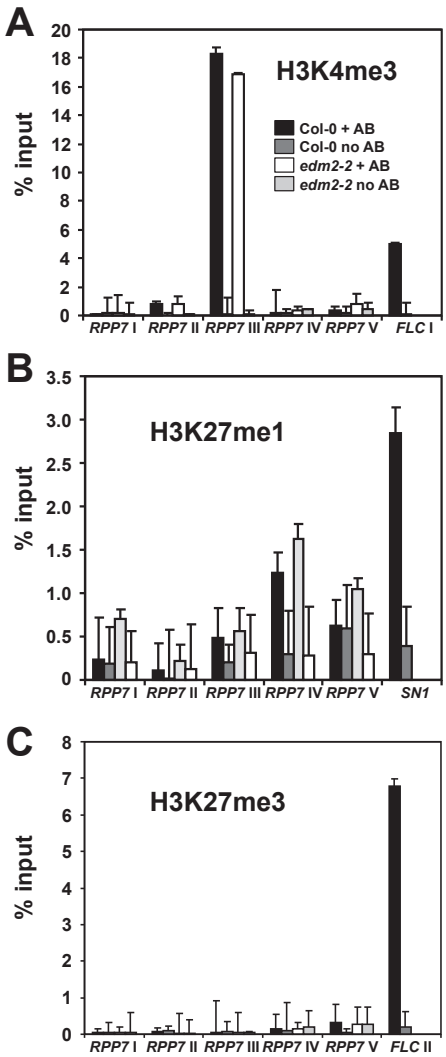

Fig. S2. Levels of H3K4me3, H3K27me1, and H3K27me3 at *RPP7* determined by ChIP. ChIP-qPCR to measure levels of H3K4me3 (*A*), H3K27me1 (*B*), and H3K27me3 (*C*) at *RPP7*. Five different *RPP7* regions marked in Fig. 1*A* were tested. Regions of *FLC* (*FLOWERING LOCUS C*; AT5G10140) and *SN1* (a short interspersed nuclear retroelement) served as positive controls. ChIP was performed with antibodies against histone modifications (+ AB) or no antibody controls (no AB). Signals from the ChIP assays are represented as percentage of input. The *y* axes represent % input. Error bars represent SEM for two biological replicates with three technical replicates each.

```
COPIA-R7 5'-LTR      1 TATCAACACAATACAGGTAAGAGCAGAGGCTTTCTCTGGCAAACGACAATACGTTGTCGT    60
COPIA-R7 3'-LTR      1 TATCAACACAATACAGGTAAGAGCAGAGGCTTTCTCTGGCAAACGACAATACGTTGTCGT    60
                       ************************************************************

COPIA-R7 5'-LTR     61 TTCGGCTAATGGAGCAAAGAGGAAAATGAAACAGATGGGCCTCGCACAAACCAAGCCCAA   120
COPIA-R7 3'-LTR     61 TTCGGCTAATGGAGCAAAGAGGAAAATGAAACAGATGGGCCTCGCACAAACCAAGCCCAA   120
                       ************************************************************

COPIA-R7 5'-LTR    121 GAAGCAGA------GGCAACGGTCATCTGTTTCAAGTAAACAGAGTCTAGAATCTTCCTC   174
COPIA-R7 3'-LTR    121 GAAGCAGAAGTGGAGGCAACGGTCATCTGTTTCAAGTAAACAGAGTCTAGAATCTTCCTC   180
                       ********      *******************************************

COPIA-R7 5'-LTR    175 AAGTAATGACAAAAGTGGAGCTGGCAATAAGGAGACAACGACCTCTTGTACGGCAAAGCA   234
COPIA-R7 3'-LTR    181 AAGTAATGACAAAAGTGGAGCTGGCAATAAGGAGACAACGACCTCTTGTACGGCAAAGCA   240
                       ************************************************************

COPIA-R7 5'-LTR    235 GTTCAACGTAAAGCTCGAGAATCGTTTCTCAACCCTTGATTCAGAAGACTCAACTTGATG   294
COPIA-R7 3'-LTR    241 GTTCAACGTAAAGCTCGAGAATCGTTTCTCAACCCTTGATTCAGAAGACTCAACTTGATG   300
                       ************************************************************

COPIA-R7 5'-LTR    295 TATGACATCTGTCCAATGTCTCTAGGGTTTTAGCTAAGTCTCCACCTTTGTATTTAAGTA   354
COPIA-R7 3'-LTR    301 TATGACATCTGTCCAATGTCTCTAGGGTTTTAGCTAAGTCTCCACCTTTGTATTTAAGTA   360
                       ************************************************************

COPIA-R7 5'-LTR    355 TTCTCAAACTCAAGTTGTAAGCTTTAAGCAAACTATCAATGAAAACCTAAAAAGATTGAT   414
COPIA-R7 3'-LTR    361 TTCTCAAACTCAAGTTGTAAGCTTTAAGCAAACTATCAATGAAAACCTAAAAGGATTGAT   420
                       ************************************************** *******

COPIA-R7 5'-LTR    415 TTTGTCTTCA                                                   424
COPIA-R7 3'-LTR    421 TTTGTCTTCA                                                   430
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Identity = 98.1%

Fig. S3. Alignment of 5′- and 3′-LTR nucleotide sequences of *COPIA-R7*. Identical nucleotides are represented by asterisks (*). Dashes (–) indicate gaps.

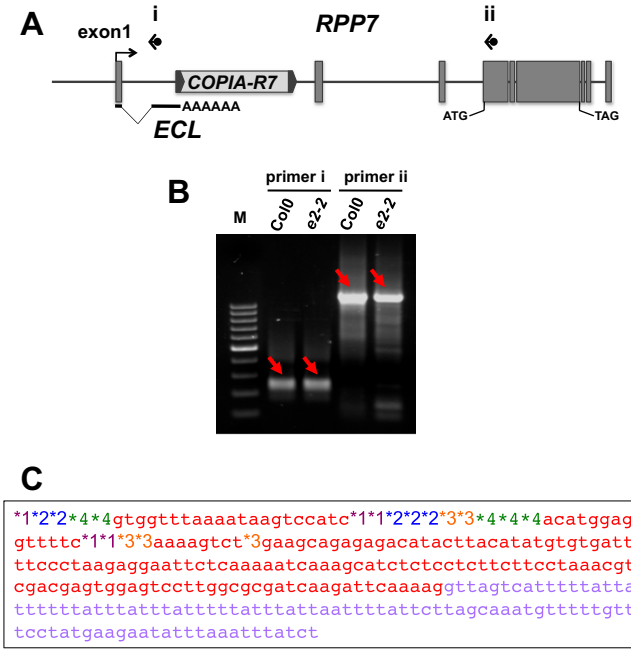


Fig. S4. ECL and the RPP7-coding mRNA share a cluster of common TSSs. (*A*) Schematic representation of the *RPP7* locus in *Arabidopsis* Col-0 accession. Black horizontal arrows represent PCR primers used for 5′RACE (i and ii). (*B*) The 5′RACE analysis of poly(A)-selected total mRNAs extracted from Col-0 or *edm2-2* mutant plants with primers represented in *A*. The bands marked with red arrows were further analyzed by sequencing. M, GeneRuler 100 bp DNA ladder (Thermo Scientific). (*C*) Nucleotide sequences at the region of transcription start sites of ECL and RPP7-coding transcripts. Exon and intron sequences are colored by red or light blue, respectively. The TSSs revealed by sequencing of cloned 5′RACE products in *B* are indicated by asterisks with numbers. *1, TSSs of ECL in Col-0; *2, TSSs of ECL in *edm2-2*; *3, TSSs of RPP7-coding transcripts in Col-0; *4, TSSs of RPP7-coding transcripts in *edm2-2*.

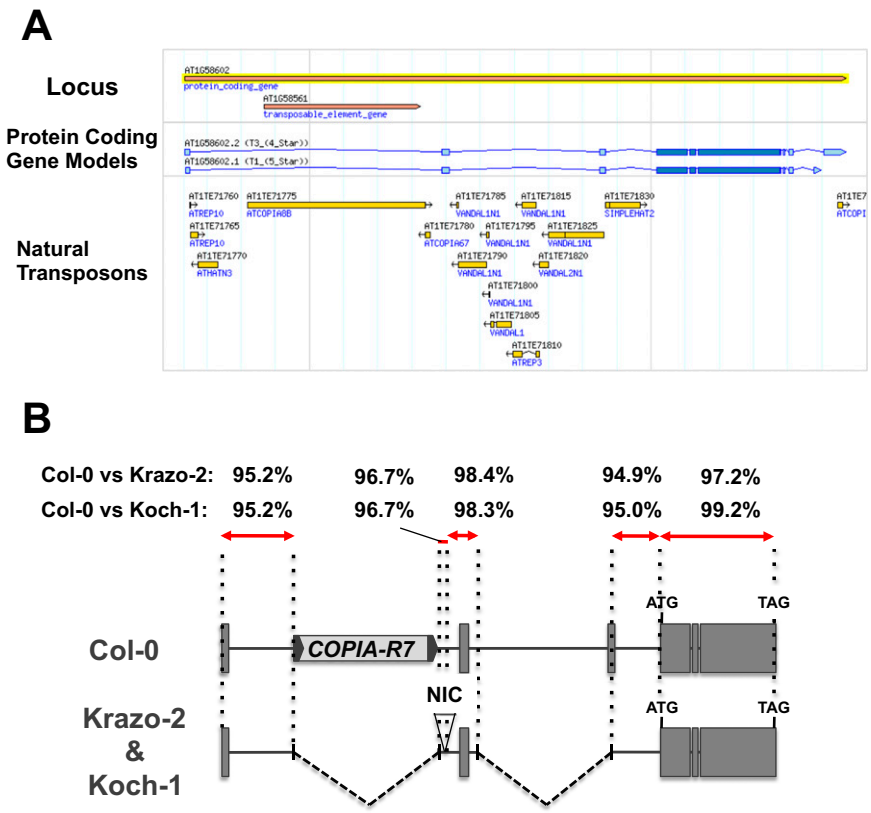
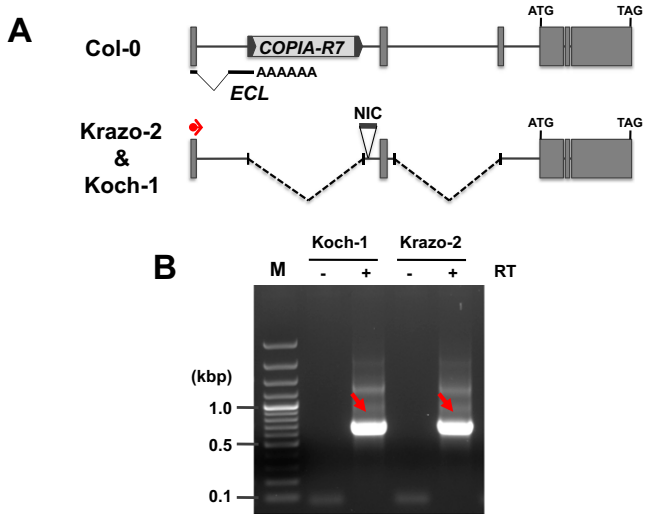


Fig. S5. Genomic structure of *RPP7* orthologs in Krazo-2 and Koch-1. (*A*) Gene structure of *RPP7* in Col-0. Locus: AT1G58602 is *RPP7*; AT1G58561 is *COPIA-R7*. Protein coding gene models: spliced mRNA forms of RPP7. Natural transposons: predicted transposable elements (1). All data are obtained from The *Arabidopsis* Information Resource (TAIR; www.arabidopsis.org/index.jsp). (*B*) Comparison of genomic structures of Col-0 *RPP7*, $RPP7_{Krazo-2}$, and $RPP7_{Koch-1}$. Numbers indicate % identity of the nucleotide sequences in each region represented by red arrows.

1. Buisine N, Quesneville H, Colot V (2008) Improved detection and annotation of transposable elements in sequenced genomes using multiple reference sequence sets. *Genomics* 91(5): 467–475.



C

NIC start

```
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tggtttggtttggtttggtttgtatacttaggaaataggaagtaacctgataac
cgaaatacatttggttatggttcggttcatataactatatacccaaaataaccg
aagttaccgaactttggttaaaatatttacggtcatgtcttaacggttgtgtct
ttttcatatcttttctttaaaatattgtatcgttttgttaaattatcatatttt
ttggt*1aaactttaggt*2aataattcaaatatttgaatatgttatcacaata
attttattaataaatactatatctgagcgttcgtttc*3ta*4ttccagttatt
tatccaaaatatccaaaaagaactgaaagactaacatcctaaacctttttgaca
aaattgtgatgggcaaaatacc*5aaaaaattaacaaaacattattgggttaga
ggttggggcaaattctatttggcttagatgtagttttgtaaagagttgaccaaa
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tatacccaatatcaatggatactagatagtgtgagtgaattcattcgggctatt
tagtggt
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NIC end

D

NIC start

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tcaaaataattttgagttgtttttggtttcgttcggtttgcgtagcgctcggtt
tggtttggtttggtttggtttgtatacttaggaaataggaagtaacctgataac
cgaaatacatttggttatggttcggttcatataactatatacccaaaataaccg
aagttaccgaactttggttaaaatatttacggtcatgttttaacggttgtgtct
ttttcatatcttttctttaaaatattgtatcgttttgtt*1aaattatcatatt
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tatacccaatatcaatggatactagatagtgtgagtgaattcattcgggctatt
tagtggt
```

NIC end

Fig. S6. Polyadenylation sites of $ECL_{Krazo-2}$ and ECL_{Koch-1}. (*A*) Schematic representation of Col-0 *RPP7* and its orthologous loci of Krazo-2 and Koch-1. Red horizontal arrow represents the PCR primer used for 3′RACE. (*B*) The 3′RACE analysis of poly(A)-selected total mRNAs extracted from Koch-1 or Krazo-2 with the primer represented in *A*. The 3′RACE was performed with (+) or without (–) reverse-transcriptase (RT). The bands marked with red arrows were cloned and further analyzed by sequencing. M, GeneRuler 100 bp DNA ladder (Fermentas). (*C* and *D*) Nucleotide sequences of the sequence block of NIC in Koch-1 (*C*) or Krazo-2 (*D*). Polyadenylation sites revealed by sequencing of cloned 3′RACE products shown in *B* (five clones for each accession) are indicated by asterisks with numbers.

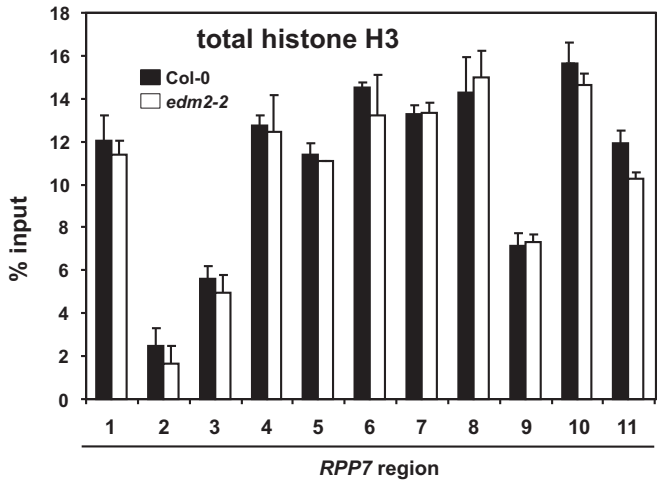

Fig. S7. Levels of total histone H3 at *RPP7* determined by ChIP. ChIP-qPCR to measure levels of histone H3 at *RPP7*. Eleven different *RPP7* regions marked in Fig. 3*A* were tested. The antibody used recognizes histone H3 regardless of modifications at the N-terminal tail of histone H3. The *y* axis represents % input. Error bars represent SEM for two biological replicates with three technical replicates each.

Table S1. Oligonucleotides used in this study

Name	Sequences (5′→3′)	Comments
	For transcript measurements	
RPP7 unsp RT F1	TTGGCGCGATCAAGATTCAA	Unspliced RPP7 transcripts. Combination a in Fig. 1*A*.
RPP7 unsp RT R1	TAGGAATGCTGTTCATTTTACAAGA	Unspliced RPP7 transcripts. Combination a in Fig. 1*A*. Used for reverse-transcription
COPIA-R7 RT F	CTCACAAACGGATCTCCTAACCTA	COPIA-R7 transcripts. Combination b in Fig. 1*A*.
COPIA-R7 RT F	GCTCCCGGAAAACTGAACTGA	COPIA-R7 transcripts. Combination b in Fig. 1*A*.
RPP7 coding RT F	ACGTGAACTATATCAATTGTTGGAA	RPP7-coding transcripts. Combination c in Fig. 1*A*. Also used in Fig. 6 *A*, *B*, and *D*.
RPP7 coding RT R	TTTGTAGGCGCAACGATACTCT	RPP7-coding transcripts. Combination c in Fig. 1*A*. Also used in Fig. 6 *A*, *B*, and *D*.
EDM2 RT F	CTTGGACCCCATGTTAGCTC	For EDM2 transcripts
EDM2 RT R	TTAAGAGATTATGTCCGCTAGGTT	For EDM2 transcripts
RPP7 exon1 F	ATCTCTCCTCTTCTTCCTAAACGTC	Specific to exon1 of RPP7. Primer a in Fig. 2*A*. Also used in Fig. 6*A*.
RPP7 exon1 R	TCTTGATCGCGCCAAGGACTC	Specific to exon1 of RPP7. Primer b in Fig. 2*A*. Also used in Fig. 6*A*.
RPP7 exon2 F	CAAGACTAAATAAGATAGGAAATGGGTT	Specific to exon2 of RPP7.
RPP7 exon2 R	ATACTGCTAGACGCTGGACT	Specific to exon2 of RPP7.
RPP7 exon3 F	AATTTGGCTGAATGACTTAATGGT	Specific to exon3 of RPP7.
RPP7 exon3 R	GGTAAATTATACACAGTAGCCGTTG	Specific to exon3 of RPP7.
RPP7 exon4 F	TTACCCTGAAGAATGTATGGCAAA	Specific to exon4 of RPP7.
RPP7 exon4 R	TTCCAACAATTGATATAGTTCACGTT	Specific to exon4 of RPP7.
ECL R	TGAATCCAAAGAATGAGATCCACGTA	For ECL transcripts. Used with RPP7 exon1 F. Primer c in Fig. 2*A*.
ECL F2	CATATGTGTGATTCTTCCCTAAGAGG	For ECL transcripts. Used in Fig. 6 *A* and *B*.
ECL R2	AAATCTTGCACAGAGGTCCTGA	For ECL transcripts. Used in Fig. 6 *A* and *B*.
ACT8 RT F	CAGTGTCTGGATTGGTGGTTCTATC	ACTIN8 internal control.
ACT8 RT R	ATCCCGTCATGGAAACGATGT	ACTIN8 internal control.
	For ChIP-qPCR	
RPP7 ChIP I F	CCAGCCCATTTTAAACCTGT	Region I in Fig. 1*A* and Fig. S3. Region 1 in Fig. 3*A*.
RPP7 ChIP I R	GAAGAGTTTATCCGTTATCTATTCGT	Region I in Fig. 1*A* and Fig. S3. Region 1 in Fig. 3*A*.
RPP7 ChIP II F	ACTATGCCAATAATTATAGTAGCCGAC	Region II in Fig. 1*A* and Fig. S3. Region 2 in Fig. 3*A*.
RPP7 ChIP II R	AGCCTTACTTTGAATTATCTTGATTCGT	Region II in Fig. 1*A* and Fig. S3. Region 2 in Fig. 3*A*.
RPP7 ChIP III F	AATGAACAGCAACTAGAGGCA	Region III in Fig. 1*A* and Fig. S3. Region 1 in Fig. 5*B*. Region 3 in Fig. 3*A*.
RPP7 ChIP III R	AATACCCAATCCGAACTTGAACG	Region III in Fig. 1*A* and Fig. S3. Region 1 in Fig. 5*B*. Region 3 in Fig. 3*A*.
RPP7 ChIP IV F	AAACATCTCAAATTGTGTCACCGT	Region IV in Fig. 1*A* and Fig. S3. Region 7 in Fig. 3*A*.
RPP7 ChIP IV R	ATCGATGTCGGAGACCACACT	Region IV in Fig. 1*A* and Fig. S3. Region 7 in Fig. 3*A*.
RPP7 ChIP V F	CTTACTTGCATTGTTCCAGATCGC	Region V in Fig. 1*A* and Fig. S3. Region 11 in Fig. 3*A*.
RPP7 ChIP V R	TAGGAAATGTTCGTCGTATCTCCC	Region V in Fig. 1*A* and S3. Region 11 in Fig. 3*A*.
RPP7 ChIP 4 F	TCGCCGATCTACTTTCTAGTCAGGA	Region 4 in Fig. 3*A*.
RPP7 ChIP 4 R	CTCAAGTAGTGTACCGCCTCA	Region 4 in Fig. 3*A*.
RPP7 ChIP 5 F	AATAGTGTGATTAGCTTCTCCGTT	Region 5 in Fig. 3*A*. Region 2 in Fig. 5*B*.
RPP7 ChIP 5 R	CCTACAAACACTGGTTATATCTGGC	Region 5 in Fig. 3*A*. Region 2 in Fig. 5*B*.
RPP7 ChIP 6 F	CCCAAGAAGCAGAGGCAACG	Region 6 in Fig. 3*A*. Region 3 in Fig. 5*B*.
RPP7 ChIP 6 R	TTCTGAATCAAGGGTTGAGAAACGA	Region 6 in Fig. 3*A*. Region 3 in Fig. 5*B*.

Table S1. Cont.

Name	Sequences (5′→3′)	Comments
RPP7 ChIP 8 F	TCAACAACATATCTCATGCCCTC	Region 8 in Fig. 3*A*.
RPP7 ChIP 8 R	AGGAGATTAATCAGAAAATTCGCAGT	Region 8 in Fig. 3*A*.
RPP7 ChIP 9 F	CCACTTACTTCAGGAACATCGC	Region 9 in Fig. 3*A*.
RPP7 ChIP 9 R	TGATGTATCTTAGAATACGCTTCAGG	Region 9 in Fig. 3*A*.
RPP7 ChIP 10 F	CAAGACTAAATAAGATAGGAAATGGGTT	Region 10 in Fig. 3*A*.
RPP7 ChIP 10 R	TTGATACTGCTAGACGCTGGAC	Region 10 in Fig. 3*A*.
NV ECL ChIP i F	AATGAACAGCAACTAGAGGCA	ChIP in Krazo-2 and Koch-1. Region i in Fig. 5*B*.
NV ECL ChIP i R	AATACCCAATCCTAACTTGAACGG	ChIP in Krazo-2 and Koch-1. Region i in Fig. 5*B*.
NV ECL ChIP ii F	AATAGTGTGATTAGCTTCTCCGTT	ChIP in Krazo-2 and Koch-1. Region ii in Fig. 5*B*.
NV ECL ChIP ii R	CCTACAAACACTGGTTATATCTGGC	ChIP in Krazo-2 and Koch-1. Region ii in Fig. 5*B*.
NV ECL ChIP iii F	AAAATTGTGATGGGCAAAATACCAA	ChIP in Krazo-2 and Koch-1. Region iii in Fig. 5*B*.
NV ECL ChIP iii R	CTATGGATACCCGAATAGAACCAA	ChIP in Krazo-2 and Koch-1. Region iii in Fig. 5*B*.
FLC ChIP I F	GAACCCAAACCTGAGGATCA	Positive control for H3K4me3 in Fig. S3*A*. Specific to a region in *FLOWERING LOCUS C* (AT5G10140)
FLC ChIP I R	GGATGCGTCACAGAGAACAG	Positive control for H3K4me3 in Fig. S3*A*. Specific to a region in *FLOWERING LOCUS C* (AT5G10140)
FLC ChIP II F	CGCCCTTAATCTTATCATCGTT	Positive control for H3K27me3 in Fig. S3*C*. Specific to a region in *FLOWERING LOCUS C* (AT5G10140)
FLC ChIP II R	TTTTCAATTTCCTAGAGGCACCA	Positive control for H3K27me3 in Fig. S3*C*. Specific to a region in *FLOWERING LOCUS C* (AT5G10140)
A122	CCAGAAATTCATCTTCTTTGGAAAAG	Positive control for H3K27me1 in Fig. S3*B*. Specific to *SN1* (1)
A123	GCCCAGTGGTAAATCTCTCAGATAGA	Positive control for H3K27me1 in Fig. S3*B*. Specific to *SN1* (1)
Mu1 ChIP F	ACATTAGTCTGGAAATTGGCTT	Positive control for H3K9me2.
Mu1 ChIP R	CAACTACCGAGCTTATAGAAACACC	Positive control for H3K9me2.
ACT8 ChIP F	CTAAAGAGACATCGTTTCCATGACGG	Negative control for H3K9me2.
ACT8 ChIP R	TCCTTAGACATCTCTCCAAACGC	Negative control for H3K9me2.
	For RACE	
Col0 3′RACE 1–1	GCATCTCTCCTCTTCTTCCTAAACGTC	3′RACE in Col-0. Primer i in Fig. 2*A*.
Col0 3′RACE 1–2	AGTCCTTGGCGCGATCAAGATTCAA	Nested PCR for products with Col0 3′RACE 1–1.
Col0 3′RACE 2–1	TTGGCATTCCACTTCCAAACACTCC	3′RACE in Col-0. Primer ii in Fig. 2A.
Col0 3′RACE 2–2	TCTTACTGCAAACCCTGCCTTTCACG	Nested PCR for products with Col0 3′RACE 2–1.
Col0 3′RACE 3–1	ACATGTTGACATTGAGAAGTGATGATGC	3′RACE in Col-0. Primer iii in Fig. 2*A*.
Col0 3′RACE 3–2	GCAATTGCATATAGAACTTAACCGTAACCCTCT	Nested PCR for products with Col0 3′RACE 3–1.
Col0 3′RACE 4–1	GGAAATGGGTTGATACAAAGAAGTTGTCC	3′RACE in Col-0. Primer iv in Fig. 2*A*.
Col0 3′RACE 4–2	CAGCGTCTAGCAGTATCAAGCAACTC	Nested PCR for products with Col0 3′RACE 4–1.
Col0 5′RACE 1–1	ACCACCAGCGAGGAATGCTT	5′RACE for *ECL* transcripts in Col-0. Primer i in Fig. S2*A*.
Col0 5′RACE 1–2	GAGTCCTCTCCAGTCACGGCAA	Nested PCR for products with Col0 5′RACE 1–1.
Col0 5′RACE 2–1	TCCTTCTTCCATATGTCGTCAAGGACAATTA	5′RACE for RPP7-coding transcripts in Col-0. Primer ii in Fig. S2*A*.
Col0 5′RACE 2–2	TTCCAACAATTGATATAGTTCACGTTGGAG	Nested PCR for products with Col0 5′RACE 2–1.
NV 3′RACE 1–1	GTTATGGCAAAGGTTTTCTCAGGG	3′RACE to detect *ECL* in Krazo-2 and Koch-1.
NV 3′RACE 1–2	TGTGTTTCTAGGTTTTCAGCGGTCA	Nested PCR for products with Natural accessions 3′RACE 1–2.

Table S1. Cont.

Name	Sequences (5′→3′)	Comments
	For genomic DNA and cDNA cloning in the natural variants	
NV gR7-1 F	CGGGGACCACTAGCCATTACTTTCTGTGAAATGTATCAAG	PCR cloning of a part of *RPP7* genomic DNA in Krazo-2 and Koch-1.
NV gR7-1 R	TGAATCCAAAGAATGAGATCCACGTA	Used with NV gR7-1 F.
NV gR7-2 F	CTTAAGCATTCCTCGCTGGT	PCR cloning of a part of *RPP7* genomic DNA in Krazo-2 and Koch-1.
NV gR7-2 R	ATACTGCTAGACGCTGGACT	Used with NV gR7-2 F.
NV gR7-3 F	CAAGACTAAATAAGATAGGAAATGGGTT	PCR cloning of a part of *RPP7* genomic DNA in Krazo-2 and Koch-1.
NV gR7-3 R	TAGGAAATGTTCGTCGTATCTCCC	Used with NV gR7-3 F.
NV gR7-4 F	CATCATATAAAACGTTCCCATGCAA	PCR cloning of a part of *RPP7* genomic DNA in Krazo-2 and Koch-1.
NV gR7-4 R	CCAAGATACTGACAGAATAAATATGCAGA	Used with NV gR7-4 F.
NV gR7-5 F	TATTTTCTTTTTGTGATCGGCAGGT	PCR cloning of a part of *RPP7* genomic DNA in Krazo-2 and Koch-1.
NV gR7-5 R	TGGCTCTGCTGTTACATATCTCG	Used with NV gR7-5 F.
NV cR7 F	CTTACATATGTGTGATTCTTCCCTAAGAGG	Amplify *RPP7* cDNA in Krazo-2 and Koch-1.
NV cR7 R	CTGTGATTGCTGAGAGCATTCCTA	Amplify *RPP7* cDNA in Krazo-2 and Koch-1.
cEDM2 F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACAATGACGTTCGTTGACGATGA	Amplify *EDM2* cDNA in Krazo-2 and Koch-1. Contains *att*B site.
cEDM2 R	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAGTCATTAATCCAACCGCC	Amplify *EDM2* cDNA in Krazo-2 and Koch-1. Contains *att*B site.
	For the *EDM2* silencing construct	
EDM2RNAi-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTAGCGATTAAACCAATATGATCCAC	Trigger of EDM2 silencing. Contains *att*B site.
EDM2RNAi-R	GGGGACCACTTTGTACAAGAAAGCTGGGTATGATAAAGAGCCTGTTTCGT	Trigger of EDM2 silencing. Contains *att*B site.

1. Wierzbicki AT, Haag JR, Pikaard CS (2008) Noncoding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. *Cell* 135(4): 635–648.