## **Supporting Information**

## Maréchal et al. 10.1073/pnas.0901710106



**Fig. S1.** ssDNA-binding activity of AtWhy1/3 in *Arabidopsis* ptWhirlies mutants. EMSA was performed by using 20  $\mu$ g of crude plastid proteins isolated from plants of the indicated genotypes and a radiolabeled probe of 32 nt (5'-TGTCATTTTGTCATTTTGTCATTTTGTCA-3'). As a control, an anti-AtWhy1/3 antibody ( $\alpha$ -Why1/3) or preimmune serum (PI) was preincubated with Col-0 extracts before adding the probe. Addition of the antibody eliminated the signal in the Col-0 extract, confirming that this signal corresponds to a complex between Whirlies and ssDNA.



**Fig. S2.** Colocalization of StWhy1 with chloroplast DNA. (*A*) Laser scanning confocal microscopy of StWhy1-GFP tobacco leaf guard cells. (*Upper Left*) Chlorophyll autofluorescence pseudocolored in red. (*Upper Right*) GFP fluorescence pseudocolored in green. (*Lower Left*) Overlay of chlorophyll and GFP fluorescence. (*Lower Right*) Corresponding phase-contrast image. (*B*) Laser scanning confocal microscopy of a tobacco leaf mesophyll protoplast transiently expressing StWhy1-GFP and stained with the DNA dye Syto85. (*Upper Left*) Chlorophyll autofluorescence pseudocolored in red. (*Upper Right*) GFP fluorescence pseudocolored in green. (*Lower Left*) Syto85 fluorescence pseudocolored in blue. (*Lower Right*) Overlay of all 3 images. Maximum projections are shown. (Scale bars: 8 μm.)

Α

В



**Fig. S3.** AtWhy1 and AtWhy3 interact with chloroplast DNA. (*A*) Position of the amplified regions on the plastid genome. (*B*) PCR amplification of ptDNA regions after immunoprecipitation on crude plastid extracts of the indicated genotypes. Inputs represent 20% of the total DNA used in the immunoprecipitation. Representative regions are shown indicating specific interaction with AtWhy1/3. (*C*) Total ptDNA after sonication was purified and used as a template for PCR. Oligonucleotides designed to amplify regions of increasing length were used to verify the efficiency of the sonication regimen. (*D*) Immunoprecipitation on total crude organelles was performed and plastid or mitochondrial DNA was assessed by PCR using oligonucleotides designed to amplify part of the plastid *psbA* or mitochondrial *atp9* genes.



Fig. S4. Restriction map of the reorganized regions in variegated lines. The red and green arrows represent the amplified regions in Var A and Var B, respectively. Probes used in DNA get blot experiments are represented as blue lines. The HindIII restriction sites are represented as vertical black lines, and the horizontal black line corresponds to ptDNA. The expected restriction fragments in bp are shown underneath the ptDNA. A gene map of this region of Arabidopsis ptDNA is presented in Fig. S9.

DN A S



**Fig. S5.** Long (16 h) and short (4 h) exposure from Fig. 2*A*. DNA gel blot (10 μg per lane) of total leaf DNA digested with HindIII and hybridized with the probes indicated below the gel. The probe numbers refer to the nucleotides of the published *Arabidopsis* chloroplast genome (8). Expected fragments from restriction analysis of Col-0 ptDNA and the size of new fragments observed in variegated lines are presented below the probes.



**Fig. S6.** Absence of subgenomic amplicons in *ZmWhy1* maize mutants. DNA gel blots (10  $\mu$ g per lane) of total leaf DNA digested with HindIII and hybridized with the probes indicated below the gel. The probe numbers refer to the nucleotides of the published maize chloroplast genome (10). Expected fragments from restriction analysis of WT ptDNA are presented below the probes. B73 inbred line was used as the source for WT DNA. For each of the 3 *ZmWhy1* mutants, a segregating green plant issued from heterozygous seeds was used as a control (g). The pale green *ZmWhy1-2* (pg), the yellow (intermediate) *ZmWhy1-2:1* (y), and the albino *ZmWhy1-1* (al) lines have been described (11). The corresponding ethidium bromide staining of total digested DNA is presented below each panel. In *E* the ptDNA level in the albino mutant was slightly lower than in the other lines where no significant difference was observed compared with the WT. To verify this, a membrane was hybridized with plastid probe 95042-10051, partially stripped and reblotted with mitochondrial probe 255542-259720 (12). The residual signal from the plastid genome is marked by an asterisk. This confirmed that the level of ptDNA was specifically diminished in the albino *ZmWhy1-1* mutant.



Fig. S6. Continued.

S A Z d



Fig. S6. Continued.

fragments

S A Z C

8906 bp (61001-52095) 4546 bp (51926-47380) 169 bp (52095-51926) 8906 bp (61001-52095) 7393 bp (69646-62253) 1252 bp (62253-61001)

1958 bp (71604-69646) 1030 bp (72634-71604) 5558 bp (83410-77852) 3901 bp (76535-72634) 1317 bp (77852-76535)



Probes Expected

fragments

DN AS

<

85072-89967

4723 bp (88133-83410) (139322-134599) 2608 bp (90741-88133) (134599-131991) 100051-105133

5037 bp (119529-114492) 3023 bp (106226-103203) 1166 bp (101223-100057) (122675-121509) 1270 bp (102493-101223) (121509-120239) 710 bp (103203-102493) (120239-119529)

Fig. S6. Continued.

105133-110101

7738 bp (114492-106333) 3023 bp (106226-103203) 421 bp (106754-106333) 107 bp (106333-106226)



270 bp (100057-99787) (122945-122675)

4283 bp (260646-256363)

Fig. S6 Continued.

DN A C



**Fig. 57.** Model for the appearance of variegation in plants lacking Whirlies. Very low levels of rearranged products are found in Col-0 plants. These products are more abundant in single Whirly KO plants. Rearranged product levels are highest in KO1/3 plants. In these plants, some rearranged molecules can reach 10–25 times the levels of the normal plastome and thus compromise the development and function of chloroplasts.

DNAS



Fig. S8. Levels of plastid 23S and 165 rRNA in *Arabidopsis* ptWhirlies mutants. Total RNA was extracted from 100 mg of mature leaf tissue from the indicated plants. Ten micrograms of purified RNA was migrated on a 1.2% agarose gel and stained with ethidium bromide.



Fig. S9 A gene map of the ptDNA region containing the amplified rearranged regions found in Var A and Var B plants was drawn by using the web-based tool 0GDRAW (9).

## Table S1. Percentage of plants showing a variegated phenotype

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Genotype	No. of plants	No. of variegated plants	% Variegation
Col-0	2,287	0	0
K01	1,367	0	0
КОЗ	2,736	0	0
KO1/3	2,879	133	4.6

Table 2. Representative recom	bination events between sh	hort direct repeats in A	Arabidopsis plants	lacking ptWhirlies
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Reaction	Band #	Plant lines	DR positions	Short direct repeat sequences	DR length and (mismatches)
A	1	KO1, KO3 KO1/3	20366 recombinant 31005	aatatatataCAAAAATTT <u>T</u> CTTTTTTTTacacttetta gttaagttatCAAAAATTTGCTTTTTTTTacacttetta gttaagttatCAAAAATTTGCTTTTTTTTtttttttagaa	(1) 18 bp (0)
A	2*	KO1/3	30300 recombinant 30827	gtcatttatgCAAAAAAAAAAAAAAtggttatgta attggtctaaCATAAAAAAAAAAtggttatgta attggtctaaCATAAAAAAAAAAagaaaaaaag	(1) 14 bp (0)
В	1	Col-0 KO1, KO3 KO1/3	50427 recombinant 69424	aattccaatcGGAGT <u>G</u> GATTGGgcaagggata tctaaaaaatGGAGTTGATTGGgcaagggata tctaaaaaatGGAGTTGATTGGatttgcacca	(1) 12 bp (0)
В	2	KO1 KO1/3	49181 recombinant 68999	tttttatttcCCCCACACCTTTTTTatataaaatt ctctaaccttCCCCACACCTTTTTTatataaaatt ctctaaccttCCCCAC <u>CA</u> CT <u>A</u> TTTTttgctaggta	(0) 15 bp (3)
В	3	КОЗ	49972 recombinant 70402	gttttttttaCTTTTTTTTTTTTTTTAttattgtatc cccaaagtgtCTTTTTTTTTTTTTTTAttattgtatc cccaaagtgtCTTTTTTTTTTTTTTTAcggtgtgaaa	(0) 15 bp (0)
В	4	KO1/3	48553 recombinant 68998	$tagaattgtaTCCCCCCTTCATTTATTGCTttccgatctt\\ actctaacctTCCCCCCCTTCATTTATTGCTttccgatctt\\ actctaacctTCCCCACCACTATTTTTTGCTaggtattttc\\ actctaacctTCCCCACCACTATTTTTTGCTaggtattttc\\ actctaacctTCCCCACCACTATTTTTTGCTaggtattttc\\ actctaacctTCCCCACCACTATTTTTTTTGCTATTTCCTATTTTTTTTT$	(0) 21 bp (5)
С	1	KO3 KO1/3	59043 recombinant 69807	tttttgtaccTATTTTTTTATTctatttctat ttctcttcaaTATTTTTTTATTctatttctat ttctcttcaaTATTTTTTTATTtttatattga	(0) 12 bp (0)
С	2	KO1/3	59287 Recombinant 70228	acttaccctcTATTTTTGTGCCTTtagtaggcct ccgtacaggcTTTTTTTGTGCCTTtagtaggcct ccgtacaggcTTTTTTTGTGCATTgcatacggct	(1) 14 bp (1)
С	3	KO1/3	59028 recombinant 70225	tatttagtttGGCTTTTTTTGTacctatttt taaccgtacaGGCTTTTTTGTacctatttt taaccgtacaGGCTTTTTTTGTgcattgcata	(0) 12 bp (0)
С	4	KO1/3	58721 recombinant 70186	attagactagACAAACAAAAAAAAAgttcattttc agacgggataACAAACAAAAAAAAgttcattttc agacgggataACAAA <u>A</u> AAAAAAAAatagataaat	(0) 14 bp (1)

2\*: Non-specific annealing of primer 20481REV at positions 30367-30346 yielded this product.

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Table S3. Short direct repeat-mediated recombination frequency in various Arabidopsis genotypes

Plant line	PCRs, n	Total events, <i>n</i>
Col-0	30	2
KO1	30	7
КОЗ	30	6
KO1/3	30	40

## **Other Supporting Information Files**

SI Appendix (PDF) Table S4 (PDF) Table S5 (PDF) Table S6 (PDF)

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