Supporting Information

Séguéla-Arnaud et al. 10.1073/pnas.1423107112



Fig. S1. Schematic representation of the TOP3α, RECQ4A, and RECQ4B protein domains. (A) Schematic representation of TOP3α domains. The position of the top3a-R640X mutation is shown. (B) Schematic representation of RECQ4A domains. The positions of the msh4(s)84 and msh4(s)101 mutations are shown. (C) Schematic representation of RECQ4B domains. The position of the natural mutation of the Landsberg strain is shown.



Fig. 52. top3a-2 but not top3a-R640X confers a growth defect and sterility. Seven-week-old plants shown left to right: wild type, homozygote top3a-2 showing reduced growth and sterility, heterozygote top3a-2/top3a-R640X, and homozygote top3a-R640X.



Fig. S3. Average number of ring bivalents (dark blue), rod bivalents (light blue), and pairs of univalents (red) per male meiocyte. The number of cells analyzed is indicated in parentheses. The four genotypes are in the Ler-0 background. *msh4(s)84* and *msh4(s)101* are two allelic *recq4a* mutants.



Fig. 54. The *RECQ4A RECQ4B* duplication is specific to *Brassicaceae*. Although *Brassicaceae* species have both RECQ4A and RECQ4B proteins, other plant species typically have only one RECQ4 representative. The analysis was performed on the Phylogeny.fr platform and comprised the following steps: Sequences, collected from PLAZA v2.5 and Phytosome V9.1, were aligned with T-Coffee (v6.85) using the following pairwise alignment methods: the 10 best local alignments (Lalign_pair), an accurate global alignment (slow_pair). After alignment, ambiguous regions (i.e., those that contained gaps and/or were poorly aligned) were removed with Gblocks (v0.91b). The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0 aLRT). Reliability for internal branch was assessed using the aLRT test (SH-Like). Graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3).



Fig. S5. The number of DMCI foci is not affected in recq4a recq4b or $top3\alpha$ -R640X mutants. Scatter plots of the number of DMC1 foci per zygotene cell. Wild-type siblings were used as controls. ZYP1 immunolocalization was used as a counterstain to identify zygotene stage.



Fig. S6. $top3\alpha$ -1 growth is restored equally by recq4a or recq4a recq4b mutations. Seven-week-old plants, from left to right: wild type, two $top3\alpha$ -1 recq4a double mutants, and two $top3\alpha$ -1 recq4a recq4b triple mutants. Single $top3\alpha$ -1 and $top3\alpha$ -1 recq4b plants are not viable, as previously shown (1) and as confirmed by their absence in a population segregating for the three mutations (n = 300).

1. Hartung F, Suer S, Puchta H (2007) Two closely related RecQ helicases have antagonistic roles in homologous recombination and DNA repair in Arabidopsis thaliana. Proc Natl Acad Sci USA 104(47):18836–18841.

Table S1. Number of seeds per fruit

Genotype	Average no. of seeds per fruit \pm SD		
Wild type ($n = 40$)	61.5 ± 4.9		
$top3\alpha$ -R640X (n = 40)	61.8 ± 3.5		
fancm-1 (n = 40)	64.2 ± 4.3		
$top3\alpha$ -R640X fancm-1 (n = 40)	65 ± 2.7		
Wild type ($n = 23$)	66.7 ± 6		
msh4 (n = 30)	5.1 ± 1.7		
msh4 recq4a-4 (n = 30)	5.8 ± 2.1		
$msh4 \ recq4b-2 \ (n = 30)$	3.6 ± 1.9		
msh4 recq4a-4 recq4b-2 (n = 30)	58.8 ± 4.8		
recq4a-4 recq4b-2 (n = 23)	65.6 ± 5.6		
Wild type ($n = 40$)	53.7 ± 4.8		
recq4a-4 recq4b-2 fancm-1 (n = 40)	49.2 ± 5.3		

The number of fruit counted in each genotype is indicated in parentheses. Wild-type siblings were used as controls.

Table S2. Proteins identified following TOP3 TAP

AGI code	Protein name	C-terminal tag fusion, trial 1	C-terminal tag fusion, trial 2	N-terminal tag fusion, trial 1	N-terminal tag fusion, trial 2	Total*
AT5G63920	ΤΟΡ3α	1	1	1	1	4
AT5G63540	RMI1	1	1	1	1	4
AT1G08390	RMI2		1	1	1	3
AT1G56260	TEN1	1		1	1	3
AT1G10930	RECQ4A			1	1	2

*Total from four experiments.

Dataset S1. Recombination raw tetrad dataset

Dataset S1

PNAS PNAS

The FTL system tracks the segregation of transgenic markers by cyan, yellow, or red fluorescence of pollen grains within meiotically related tetrads. Drawings above each column represent the different distribution possibilities of markers among the four chromatids and the corresponding distribution of colors in the tetrad, according to the nomenclature of Berchowitz et al. (1). The observed number of each type of tetrad is given for each pair of intervals (e.g., I1bl1c are two adjacent intervals on chromosome 1) and for each genotype.

1. Berchowitz LE, Copenhaver GP (2008) Fluorescent Arabidopsis tetrads: A visual assay for quickly developing large crossover and crossover interference data sets. Nat Protoc 3(1):41–50.

Dataset S2. Mass spectrometry data from TAP experiments

Dataset S2