ROOT HAIR DEFECTIVE3 Family of Dynamin-Like GTPases Mediates Homotypic Endoplasmic Reticulum Fusion and Is Essential for Arabidopsis Development^{1[W][OPEN]}

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RESULTS

Characterization of an RHD3 Deletion Mutant

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Figure 1. Characterization of *rhd3-8*, a null mutant of *RHD3*. A, Schematic diagram of the genomic locus of *RHD3*. Exons and introns are shown in boxes and lines, respectively. Mutation sites for *rhd3-8* and *rhd3-1* are indicated. B, The transcription levels of *RHD3* in wild-type (Columbia [Col-0]), *rhd3-8*, and *rhd3-1* plants were analyzed by reverse transcription and real-time PCR. *EF1* α was used as an internal control. The data represent means \pm sp from three replicates. C, The protein levels of RHD3 in wild-type (Col-0), *rhd3-1* plants were determined by immunoblotting (IB) with anti-RHD3 antibodies (top panel). Coomassie blue staining of the RuBisCO subunit (RbcS) served as a loading control (bottom panel). D, Mature plants of Col-0, *rhd3-8*, and *rhd3-1*

D3 rhd3-8 (. 1C). d rhd3-8 RHD3. d d d RHD3 d d 1990; ., 2012), rhd3-8 ., 1997; d d d d . 1, D d rhd3-8 d,). (-D A RHD3 (A 106309; d rhd3-7; RHD3 ., 2012), d d. rhd3-7, d d RHD3 d d RHD3 d rhd3-7 d (A 047559; rhd3-9; -D A 2A). fi d d D3 d d d ,

RHD3 " 1" () ., 2004; ., 2012), rhd3-8 (C d ., 2000) d d d D D d 35 D3 . 1). (d d rhd3-8 RHD3, D3 d d d 2002) d 100 1 d d B). 3, A D3 d d 3C). d (d dd D3 d С rhd3-8 d d D3. RHD3 fi d d rhd3-1 d d A -575 d ., . 1A; 2B). rhd3-8, (rhd3-1 d RHD3 (1B). D3 d rhd3-1 (A575 1C). , , d d D3. , rhd3-1 . 1D). d D3 (d rhd3-1, d А d

RHD3 Proteins Are Essential for Plant Development

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	D3	~ ~	d	d	

RL1 RL2 d d (C 2011) d d B), 4 Α А D3, d D3 d d А d rhd3-8 -D A

Α

В

Constune	rhd3 ^{-∕-}			rl2 [≁]		
Genotype	rl1 ^{+/+}	rl1 ^{+/-}	rl1 [⊄]	rhd3 ^{+/+}	rhd3 ^{≁∕-}	rhd3 ^{-/-}
Number of plants	82	71	0	53	40	4

rhd3^{-/-}rl2^{+/-} rhd3^{-/-}rl2^{-/}

rhd3^{-⁄-}



Figure 2. Genetic interactions between *RHD3* and *RLs*. A, *rhd3* was crossed with either *rl1* or *rl2*. F3 seedlings were genotyped and counted. B, Mature plants of *rhd3^{-/-}*, *rhd3^{-/-}*, *rl2^{+/-}*, and *rhd3^{-/-}*, *rl2^{-/-}*. C, The flowers of the wild type (Col-0), *rhd3^{-/-}*, *rl2^{+/-}*, and *rhd3^{-/-}*, *rl2^{-/-}* were imaged. The bottom panels show enlarged views. Note that *rhd3^{-/-}*, *rl2^{-/-}* has no visible pollen grains. Bars = 1 mm. D, The anthers of the wild type (Col-0) and *rhd3^{-/-}*, *rl2^{-/-}* were collected at stage 12 (left panels) and 13 (middle panels) and were imaged. Pollen viability was analyzed by Alexander staining (right panels). Note that the viable pollen grains are purple and the inactive ones are green. Bars = 100 µm.

RL1 (A 048580) 116772). *RL*2 (A rhd3^{-/-} rl2^{-/-} rhd3^{-/-} rl1⁻ 2 d RL1 RL2 RHD3 d d rhd3^{-/-} rl1 rhd3^{-/-} rl2 d $rhd3^{+/-}$ rl1+/rhd3^{-/-} d rl2 d 3 d d . 2A). rhd3^{-/-} d d d (rl2 97 = 52.485 d = 5.75 -07),χ rhd3 rhd3^{-/-} d (2B). rl2^{+/-} d d rhd3 d $rhd3^{-1}$ 2B) rl2 D3 2002) rhd3^{-/-} rl2 d d d d fl . A d d 2, C d D), fl 5A). rhd3^{-/-} rl2+/ rhd3^{-/-} . 2C . 5, d В 2 d C). D3 d d rl1+/ rhd3⁻ d, d

d $rhd3^{-/-} rl1^{-/-}$ (153 rl2+/-' $d\chi$ = 88.686 = 9.24 -13). rhd3⁻ rhd3^{-/-} rl1 rhd3^{-/-} (5D). 1 D3 d fi d D3 d dd d 1 d d D3' rhd3 rls d d fi D3/ d rhd3 rl2, rhd3 rl1 $rhd3 rl2^+$ d d d rhd3-8 D3 d d d d d 6). fi d d d fi D3

RHD3 Proteins Can Functionally Replace Sey1p

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Figure 3. RHD3 proteins can replace Sey1p in yeast. A, Wild-type (wt) RHD3 proteins or the indicated mutants were expressed under the control of the endogenous SEY1 promoter in *S. cerevisiae* cells lacking Sey1p and Yop1p (*sey1* Δ *yop1* Δ cells). For expression levels, see Supplemental Figure S7. The ER was visualized by expressing Sec63-GFP, focusing the microscope on either the periphery or the center of the cells. *sey1* Δ *yop1* Δ cells expressing Sey1p or empty vector were also analyzed for comparison. Bar = 1 μ m. B, The percentage of cells with abnormal ER was determined from 80 to 200 cells per mutant.

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RHD3 Proteins Can Mediate ER Fusion in Yeast

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RHD3 Proteins Can Mediate Membrane Fusion in Vitro

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Figure 4. ER-ER fusion mediated by RHD3 proteins in yeast. A, sey 1Δ cells of opposite mating types expressing either ss-RFP-HDEL or cytosolic GFP were mixed, placed on an agarose pad, and imaged at 1-min intervals. Selected images from the time-lapse video are shown. Time 0 min is the first image taken after cell fusion, as indicated by GFP in both cells. Bar = 2 μ m. B, As in A, but with sey 1 Δ cells expressing wild-type RHD3. C, The average time between cell fusion and ER fusion during mating was determined from eight to 10 cells per sample. Values shown are means \pm sp.

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DISCUSSION

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Figure 5. RHD3 proteins mediate membrane fusion in vitro. A, RHD3, RL2, or the indicated mutants were purified and reconstituted into proteoliposomes. Flotation in a sucrose gradient (right) shows efficient reconstitution of the proteins (T, top fraction; B, bottom fraction). B, Donor (D; with NBD- and rhodamine-labeled lipids at quenching concentrations) and acceptor (A; unlabeled) proteoliposomes containing RHD3, RL2, or the indicated mutants were analyzed by SDS-PAGE and Coomassie blue staining. C, GTPase activity of full-length wild-type RHD3, RL2, or the indicated mutants was measured by phosphate release. The data represent means \pm sD from six replicates.

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MATERIALS AND METHODS

Molecular Cloning and Antibodies

RHD3, RL1, d RL2 fi d DA d-A d (Arabidopsis thaliana) fi d D A. d d RHD3 DA. (Saccharomyces cerevisiae), (A) d-D3 Ad. C2/C (A3/C d Α d d) d d d 1 C- A (2µ , . A D3 ., , , , , d). A-CA BA 1301. d D3 2 () d C--6 -1. A d C -D d () d fi d fi d D3 (d 273-DΑ d (B₁, ...). 558) d

Plant Materials

d А d В C. Ø,). rhd3-8 d A- D3 d fl d (C , 1998) Agrobacterium tumefaciensd d B , d rhd3-8 d d d 25 μ 20 C 22 C d 16- - /8- -d Ы d

Microscopy

63 d 24 d С 5 Α d . A. tumefaciens 3101 - D -5 19 600 d (D₆₀₀) 1.0 1.2. C d d d d d 200 µ fi d (10 , 10 C 2, D₆₀₀ 0.4 - 5 d 0.8 .) 0.6 - D 10 19 d 1:1 d d (/ fi 5-1-- d d. 5 7 d fi d , d d fl 4′,6-d d А d (A d $^{-1}$ 1969) d , 0.3 μ d dd d d ⁻¹ fl , 1990). A d d 165 C 0.5μ (, fl d , -fi d . , , d

In Vivo Fusion Assay

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Recombinant RHD3 and RL2 Production

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GTPase Activity Assay

d С d 100-µ d d (). 0.5 dd 360 d d 1-30 37 C., А А $(1, 2, d 5 \mu).$ d

Lipid-Mixing Assay

d d d (B ., 2011). d d d . BD d 100μ fl d 1-37 C. A ,5μ 30 β-Ddd d d 10% (/) n-D d d BD fl d fl d , . A d d a B/Bd d : D3, A 3 13870; 1, A 1 72960; 2, A 5 45160.

Supplemental Data

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Supplemental Figure S1. A d D3.	
Supplemental Figure S2. RHD3	
Supplemental Figure S3. D3 <i>rhd3-8</i> .	
Supplemental Figure S4.	
Supplemental Figure S5. rhd3 d , rl1 rl2.	
Supplemental Figure S6. RHD3 RLs d	
Supplemental Figure S7.	3.
Supplemental Figure S8. d D3	

ACKNOWLEDGMENTS

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