

(1990).
 (1997; B, 2002).
RHD3 (1990; 1997; 2012)
 D, D3, D3 (2002).
 D3 (2011).
 A, *RHD3* (2003):
 2, D, D3, D3 (2011).
 A, D3, D3 (2011).
 A, D3, D3 (2011).

RESULTS
 Characterization of an *RHD3* Deletion Mutant
 D3, *RHD3*.
RHD3 (A, 025215; *rh**d3*-8;
 -D A, *RHD3* (1A)
RHD3 (C, A, *rh**d3*-8
 A, D3 (1B).
 D3 (1).

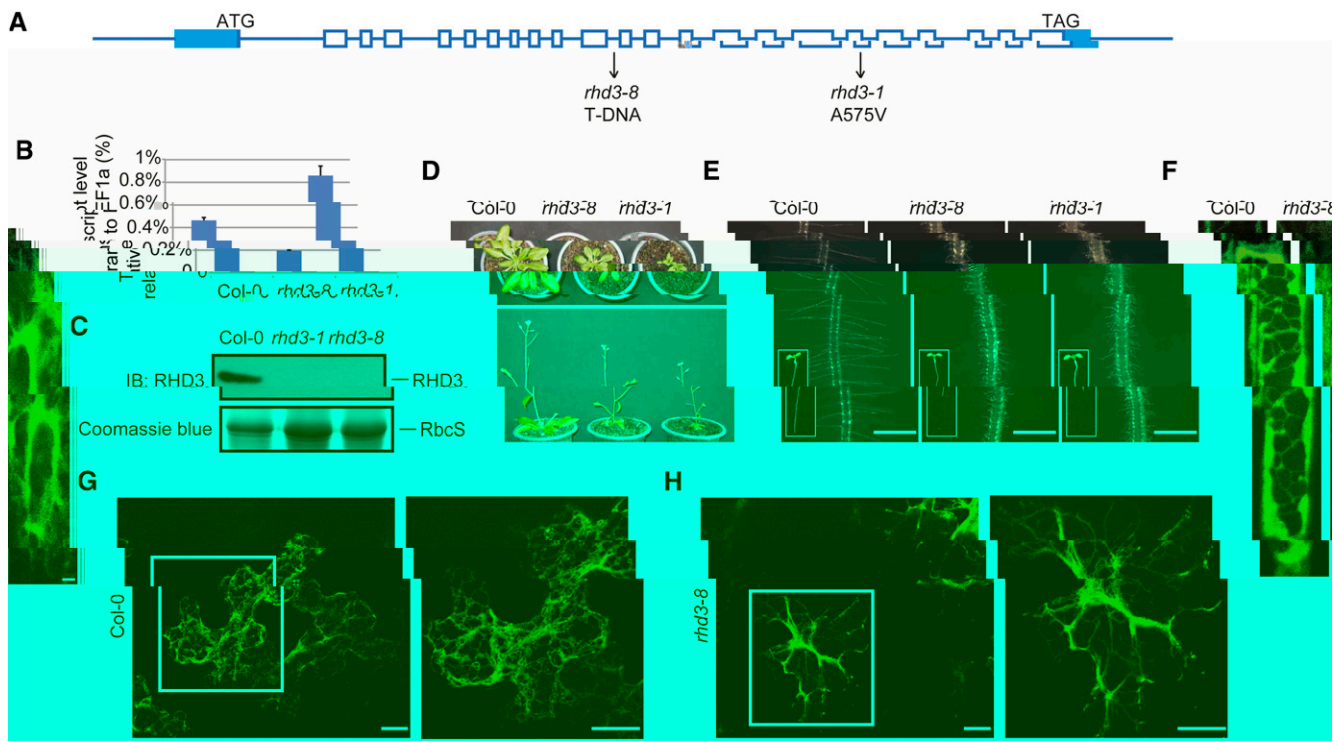


Figure 1. Characterization of *rhid3-8*, a null mutant of *RHD3*. A, Schematic diagram of the genomic locus of *RHD3*. Exons and introns are shown in boxes and lines, respectively. Mutation sites for *rhid3-8* and *rhid3-1* are indicated. B, The transcription levels of *RHD3* in wild-type (Columbia [Col-0]), *rhid3-8*, and *rhid3-1* plants were analyzed by reverse transcription and real-time PCR. *EF1α* was used as an internal control. The data represent means \pm SD from three replicates. C, The protein levels of *RHD3* in wild-type (Col-0), *rhid3-8*, and *rhid3-1* 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, *rhid3-8*, and *rhid3-1*

1 d 1 d
 1 d 1 d
 2008),
 (2009).
 d- 1 A 1 (D3
 2009; A 2012).
sey1Δyop1Δ C d- D3
 1 (7).
 (3). A d, D3 d
 D3 50A,
 (134, *rh3-5*; D185, *rh3-2*;
 A575, *rh3-1*;
 2B) d d d
 d (3).
sey1Δyop1Δ
 (3;
 D3 8C).
 1 d

RHD3 Proteins Can Mediate ER Fusion in Yeast

D3 d (A
 2012). d
 ()- (- D).
 d d
 d d
 d d
 A d d
 1 27 ; 4, A d
 C). (4C).
 D3 d d
 d- D3 d d
 (8A). d D3 fi-
 16 ; 4, B d C).
 D3 50A d (4C). 1 d
 2 (8, B d D). C -
 D3
 1

RHD3 Proteins Can Mediate Membrane Fusion in Vitro

D3 d fi d-
 d d d

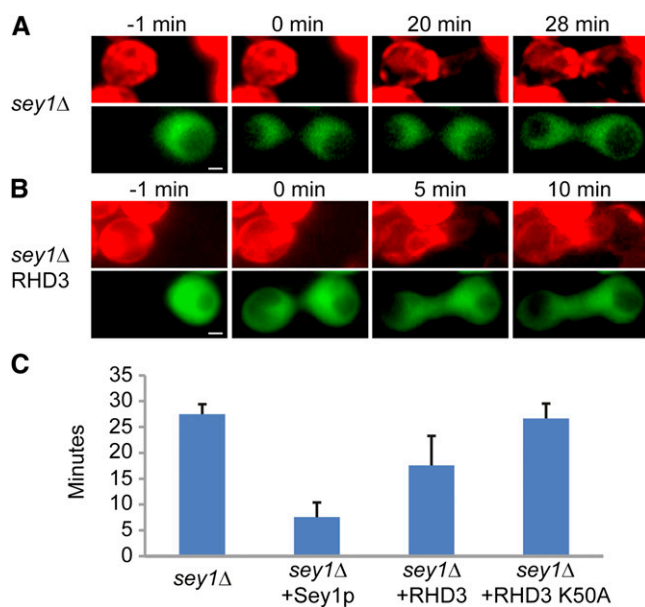


Figure 4. ER-ER fusion mediated by RHD3 proteins in yeast. A, *sey1Δ* cells of opposite mating types expressing either ss-RFP-HDEL or cytosolic GFP were mixed, placed on an agarose pad, and imaged at 1-min intervals. Selected images from the time-lapse video are shown. Time 0 min is the first image taken after cell fusion, as indicated by GFP in both cells. Bar = 2 μm. B, As in A, but with *sey1Δ* cells expressing wild-type RHD3. C, The average time between cell fusion and ER fusion during mating was determined from eight to ten cells per sample. Values shown are means ± SD.

d d d d (BD)
 d d d d ;
 d d d d fl d-
 d d d d D3 2
 d d d d S-
Escherichia coli. A d fi
 d d d d (5A).
 fi (5B). fi d fi D3 -
 d (5C). D3 d
 d- (5, D d). A d
 A d 1 d
 D d d d γ (5, D
 d). A 53A 2), d (50A
 D3 d D3
 5). d d D3

DISCUSSION

d d
 D3 d
 d D3 D3
 D3 (

d d d D3
 A d
 fi (d , 2003).
rhd3 fi d
 d d *rhd3-7* ((, 2012)
 d d *rhd3-1*, d *rhd3-8*, d
 d D3 d
 A A 1
 d *rhd3* d
 -d d
 (, 2009, 2011).
 d d
 d d fi d

MATERIALS AND METHODS

Molecular Cloning and Antibodies

RHD3, *RL1*, d *RL2* fi d D A d-
 A d (*Arabidopsis thaliana*) d d *RHD3*
 fi d D A. (*Saccharomyces cerevisiae*),
 (A3/C d (A) d- D3 A- d C2/C
 d d 1 C- A (2μ d
 A). CA B A 1301. A- D3 d D3 2
 C- () d -6 -1. A
 d fi d D A C -D d ()
 558) d (B).

Plant Materials

A d d A d B
 C *rhd3-8* A- D3 d
Agrobacterium tumefaciens- d fi d (C d B , 1998)
 d d *rhd3-8*
 25 μ⁻¹ d 20 C 22 C d 16- /8- d d

Microscopy

24 d 63 - d C 5
 3101 - D - 5 19 A d , *A. tumefaciens*
 d 600 (D₆₀₀) 1.0 1.2.C d d d d
 fi d (10 , 10 C₂ d 200 μ
) D₆₀₀ 0.4 0.6 - D - 5 d 0.8 1.0
 19 d 1:1 (/) d d
 fi 5- - d 1- d
 fi d 5 7 d
 4,6-d d A d (A d ,
 1969) d d 0.3 μ⁻¹ d d d
 0.5 μ⁻¹ fi d (d , 1990). A 165 C
 d -fi d , fi d

d d A 1. A
 Ad

In Vivo Fusion Assay

d d d (A
 , 2012).
 D D₆₀₀ 0.1 0.4 d d
 d d d 1-

Recombinant RHD3 and RL2 Production

S- d ()- d D3 2
 d *Escherichia coli* D 3 (). C
 d 37 C. d D₆₀₀ 0.6,
 d d 0.5 -β- d 12,
 24 C. d d d 50
 8.0, 500 C, 2 β- d
 (). d d 40,000
 30 d d d
 2% (/) -100 2. ()- d d
 d (), d d
 d d 2.5 d-d d-d d
 d (), d fi d
 1 -1

GTPase Activity Assay

d d C
 (dd). d d 100-μ d d
 dd 0.5 d 360 d d 1-
 d 30 37 C. d d
 (1, 2, d 5 μ).

Lipid-Mixing Assay

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

Supplemental Data

- Supplemental Figure S1. A d D3.
- Supplemental Figure S2. *RHD3*
- Supplemental Figure S3. D3 *rhd3-8*.
- 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

d A d B C d A d
 d d d
 d/j 4, 2013; d A 5, 2013; d A 6, 2013.

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