

Supplemental Dataset Chapter 3

Supplemental Methods

Generation of GUS Reporter Constructs

Promoter sequences of *ARO* genes were amplified from *Col-0* genomic DNA using the primers A2pLP/A2pRP (*ARO2*), A3pLP/A3pRP (*ARO3*) and A4pLP/A4pRP (*ARO4*) and cloned into the pENTR™D/TOPO® vector according to the manufacturer's recommendations (LifeTechnologies). To drive β -Glucuronidase (GUS) reporter expression, entry clones were recombined with pMDC206-GUS (Ulrich Hammes, unpublished) modified from pMDC206 (Curtis and Grossniklaus, 2003) by LR Clonase® reaction according to the manufacturer's protocol (LifeTechnologies).

GUS Staining Procedure

For GUS reporter expression analysis, 2-3 week old seedlings were put in GUS staining solution (0.5 mM $K_4Fe(CN)_6$, 0.5 mM $K_3Fe(CN)_6$, 1 % Triton X-100, 1 mg/ml X-Gluc (Applichem) in 50 mM sodium phosphate buffer pH 7.2), vacuum infiltrated for 10 minutes and incubated at 37 °C overnight. Clearing was performed by stepwise increasing the Ethanol concentration from 30 % to 100 % over one day.

Oligonucleotide Sequences of PCR Primers for Characterization of T-DNA Mutants

Primer Name	Nucleotide Sequence (5' to 3')
a1LP	GCTTGCACGTGACAATGATCG
a1RP	GCTTCACGAGTGGTCCAATGA
a2LP	GCTCCACGCTCTCAGGATCAC
a2RP	TTTCGCAGGTTCGGTGAATGT
a3LPsalk	CATTGGAACACACGTGGAAATCA
a3RPsalk	AACGAGGAGGAGGACGACGAT
a3LPsail	TCAACGATTCTCGGAATAACTGG
a3RPsail	ACGATGGCTACATAATGCGAC
a4LP	TGGTGGAAGATTTTTCACCAG

a4RP	CTTTCGTTTCCGTAATCCTCC
rhogapRP	GAGGTTTCTTTGGGGTTTTTG
rhogapRP	GCCATCTGGAAATAATACCG
exo70e2LP	CACTGGCGAGATAGACCTGAG
exo70e2RP	GGGAGCTTATAGTTTGGGGTG
LBb1.3	ATTTTGCCGATTTCGGAAC
LB3	CATCTGAATTCATAACCAATCTCGATACAC
GK_08409	ATATTGACCATCATACTCATTGC
ACT3LP	GATTTGGCATCACACTTTCTACAATG
ACT3RP	GTTCCACCACTGAGCACAATG

Oligonucleotide Sequences of Construct Specific PCR Primers for Genotyping and Sequencing

<i>Primer Name</i>	<i>Nucleotide Sequence (5' to 3')</i>
A1LPgen	CGATTGATGAGATTTTACCAGAAGC
A2LPgen	CGAAGCAGTCATGGGTAA
A3LPgen	GTTTTGCTGAGCTACGTG
A4LPgen	GGAGTTGAGAGAACTCGT
eGFPRP	TCAGCTTGCCGTAGGTGGCATC

Oligonucleotide Sequences of Cloning PCR Primers

<i>Primer Name</i>	<i>Nucleotide Sequence (5' to 3')</i>
A2pLP	caccCCCTTTTCTATGAGTATTTTCCAGC
A2pRP	CGTCGATATCAGATCAAATGGCG
A3pLP	caccGCAAGACTACACTATTA ACTCTG
A3pRP	GGATAACGAACTGGTTACGATCTT
A4pLP	caccCAAAAAGGATATAAGAGTATGGGG
A4pRP	GAAATCTGGTGAAAAATCTTCCACCA
A2RPmdc	TATTGAAATTGTAACCCCTTGATCC
A3RPmdc	GGGGCAAGTGAAAGCCTCTAGATC

A1pSac	GGGGGagctcgCAAGACTACACTATTAACCTCTGTG
A1pSpe	GGGGactagtGGATAACGAAACTGGTTACGATC
A3pSac	GGGGGagctcgCAAGACTACACTATTAACCTCTGTGAT
A3pSpe	GGGGactagtGGATAACGAAACTGGTTACGATCA
A1LP	caccATGGCGGATATTGTGAAACAGATC
A1RP	ATGAAATCCTCTTGACCCTCTAGATTG
A2LP	caccATGGCAGACATCGTCAAGCAAA
A2RP	ATTGAAATTGTAACCCCTTGATCCTC
A3LP	caccATGGGTGATCTAGCCAAGCAG
A3RP	CAAGTGAAAGCCTCTAGATCCTC
A4LP	caccATGGGAGAAGAAGAGAGTCG
A4RP	AGGACCAACATACGATAGC
A2LPavr	CTGAacctaggCGATGGCAGACATCGTCAAG
A2RPavr	GGATCAAAGGGTTACAATTTCAATTGcctaggTATT
eGFPLP	CACCATGGTGAGCAAGGGCGAGGAG
eGFPRP	TTACTTGTACAGCTCGTCCATGCCG
RhoGAPLP	caccATGGAGGCTTCTTTAG
RhoGAPRP	TTAGTTCCAAGGTGGAGATGAT
Exo70E2LP	caccATGGCAGAGTTTGATTCCAAGGTTC
Exo70E2RP	TCTCTTACGAGAGCTGCGCAGTGAT
RHD3LP	caccATGGATGCCGCTTGCTCTAC
RHD3RP	TGCACTTGTATCAATGGGTACG
ARO1_pGBKT7_pGADT7_X maI_fw	GTTCCCGGGCATGGCGGATATTGTGAAACAGAT
ARO1_pGBKT7_PstI_rev	TCGCTGCAGTTCAATGAAATCCTCTTGACCCTC
ARO1_pGADT7_SacI_rev	CGTGAGCTCTCAATGAAATCCTCTTGACCCTC
ARO2_pGBKT7_pGADT7_X maI_fw	AAAGGATCCATCAATTGAAATTGTAACCCCTT
ARO2_pGBKT7_pGADT7_B amHI_rev	TGACCCGGGGATGGCAGACATCGTCAAG

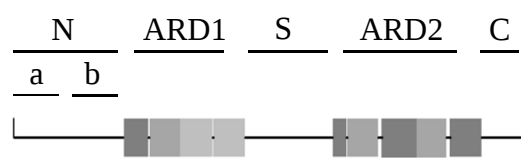
ARO3_pGBKT7_pGADT7_E coRI_for	TCCGAATTCATGGGTGATCTAGCCAAGCAG
ARO3_pGBKT7_pGADT7_Ps tI_rev	AATCTGCAGATTACAAGTGAAAGCCTCTAGATCCTC TT
ARO4_pGBKT7_pGADT7_E coRI_for	CCAGAATTCATGGGAGAAGAAGAGAGTCGAATT
ARO4_pGBKT7_pGADT7_B amHI_rev	GTAGGATCCTTTAAGGACCAACATACGATAGCATT
EXO70C1_ pGBKT7_pGADT7_NdeI_for	AAACATATGGAGAAATCTGGAAATCACC
EXO70C1_ pGBKT7_pGADT7_XhoI_rev	AAACTCGAGTTATCTCTTGCGTGCCATAGAA
EXO70E2_ pGBKT7_pGADT7_NdeI_for	AAACATATGGAGAAATCTGGAAATCACC
EXO70E2_ pGBKT7_pGADT7_XhoI_rev	AAACTCGAGTTATCTCTTGCGTGCCATAGAA
EXO70H3_ pGBKT7_pGADT7_NdeI_for	AAACATATGAGGAGCAAGAGCGGT
EXO70H3_ pGBKT7_pGADT7_XhoI_rev	AAACTCGAGTCAAAATCTTCTGCATGACAAAG
EXO70H5_ pGBKT7_pGADT7_NdeI_for	AAACATATGATGCTACTATTTAAACCGTCTTT
EXO70H5_ pGBKT7_pGADT7_XhoI_rev	AAACTCGAGTCAACGTCCTGATTCTGAATCTT

List of Constructs Used for High-Throughput Yeast Two-Hybrid Screening

<i>Construct</i>	<i>Gene Fragments (bp)</i>
<i>ARO1</i>	0001-1992
<i>ARO1_{ΔC}</i>	0001-1827
<i>ARO1_{ΔARD2,C}</i>	0001-1245
<i>ARO1_{ΔS,ARD2,C}</i>	0001-0903
<i>ARO1_{ΔNab,ARD1}</i>	0903-1992
<i>ARO2_{ΔNab,ARD1,S}</i>	1245-1992
<i>ARO1_{ΔNab}</i>	0429-1992
<i>ARO1_{ΔNab,C}</i>	0429-1827
<i>ARO1_{ΔNa}</i>	0264-1992
<i>ARO1_{ΔNa,C}</i>	0264-1827
<i>ARO1_{ΔS}</i>	0429-0903,1245-1992
<i>ARO2</i>	0001-1953
<i>ARO2_{ΔC}</i>	0001-1782
<i>ARO2_{ΔARD2,C}</i>	0001-1200
<i>ARO2_{ΔS,ARD2,C}</i>	0001-0906
<i>ARO2_{ΔN}</i>	0399-1953
<i>ARO2_{ΔN,ARD1}</i>	0906-1953
<i>ARO2_{ΔN,ARD1,S}</i>	1200-1953
<i>ARO3</i>	0001-2010
<i>ARO3_{ΔC}</i>	0001-1842
<i>ARO3_{ΔARD2,C}</i>	0001-1254
<i>ARO3_{ΔS,ARD2,C}</i>	0001-0912
<i>ARO3_{ΔN}</i>	0475-2010
<i>ARO3_{ΔN,ARD1}</i>	0913-2010
<i>ARO3_{ΔN,ARD1,S}</i>	1266-2010
<i>ARO4</i>	0001-1845
<i>ARO4_{ΔC}</i>	0001-1668

$ARO4_{\Delta ARD2,C}$	0001-1083
$ARO4_{\Delta S,ARD2,C}$	0001-0921
$ARO4_{\Delta N}$	0421-1845
$ARO4_{\Delta N,ARD1}$	0921-1845
$ARO4_{\Delta N,ARD1,S}$	1071-1845

General Structure of ARO Proteins, according to Gebert et al., (2008):



N: N-Terminus

ARD: Armadillo Repeat Domain

S: Spacer Region

C: C-Terminus

Supplemental References

- Curtis, M.D., and Grossniklaus, U.** (2003). A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol.* **133**, 462-469.
- Gebert, M., Dresselhaus, T., and Sprunck, S.** (2008). F-Actin organization and pollen tube tip growth in *Arabidopsis* are dependent on the gametophyte-specific armadillo repeat protein ARO1. *Plant Cell* **20**, 2798-2814.
- Stefano, G., Renna, L., Moss, T., McNew, J.A., and Brandizzi, F.** (2012). In *Arabidopsis*, the spatial and dynamic organization of the endoplasmic reticulum and Golgi apparatus is influenced by the integrity of the C-terminal domain of RHD3, a non-essential GTPase. *Plant J.* **69**, 957-966.
- Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., and Gruissem, W.** (2004). GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol.* **136**, 2621-2632.