Title

Arabidopsis *aux1*^{rcr1} Mutation Alters Auxin Resistant1 Targeting and Prevents Expression of the Auxin Reporter *DR5:GUS* in the Root Apex

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Supplemental Data S1. Primer sequence and the cloning of transgenes for this study.

The primer sets for qRT-PCR are CTR1-RT-F2 (5'-GCTTATCTGCAATCGTATGGG-3') and CTR1-RT-R2 (5'-GGCAATTCGACAGGGTAAAT-3') for *CTR1*, AUX1-RT-wR (5'-GAACCAAGTAATCCATCAAG-3') and AUX1/rcr1-RT-F (5'-CAGGAATAGTACTTCAGATC-3') for *AUX1*, and rcr-RT-mR (5'-GAACCAAGTAATCCATCAAA-3') and AUX1/rcr1-RT-F (5'-CAGGAATAGTACTTCAGATC-3') for *aux1*^{rcr1}. Gene expression measurement was presented as mean±SE (standard error for the mean of three measurements of three independent biological samples). The primer sets for genotyping are CTR1-LB (5'-AGTGGACTCTTGTTCCAAACTG-3') and ctr1-10-R (5'-CCACTGGATTGCCTTTGCAACC-3') for *ctr1-10*, AUX1-T-R (5'-GACAGTGGTTGCACAACACG-3') and aux1-LB (5'-AGTGGACTCTTGTTCCAAACTG-3') for *aux1-T*, CTR1-F (5'-GGATCCAATGTTACGGATTCAG-3') and ctr1-10-R (5'-CCACTGGATTGCCTTTGCAACC-3') for *CTR1*, and AUX1-F (5'-TGACAACGGAACAGATCAGGT-3') and AUX1-R (5'-GACAGTGGTTGCACAACACG-3') for *AUX1*. For the genotyping of *aux1*^{rcr1}, the primes set AUX1-RsaI-F (5'-TCAGATCTTCTATGGTTTACTAGG-3') and AUX1-RsaI-R (5'-GGTGTAAGTGGTCATTCCAAGG-3') generated an *AUX1* fragment by PCR, and the wild-type but not *aux1*^{rcr1}fragment was restricted by *Rsa*I.

Cloning of transgenes and *Agrobacterium*-mediated transformation

To clone *AUX1*, genomic DNA from the wild-type (Col-0) plant was used as the PCR

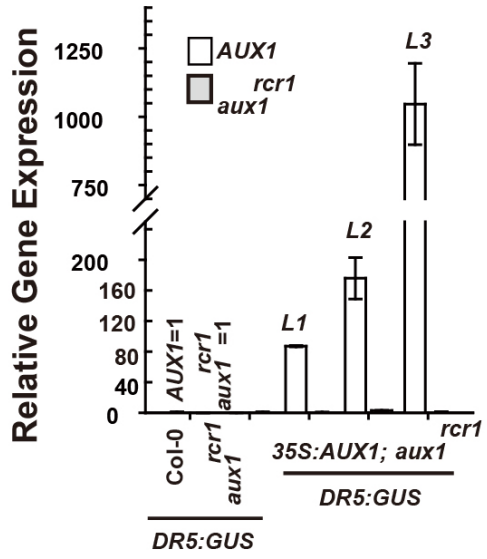
template. The primer sets AUX1-ATG-F-KpnI (5'-CCAGGTACCATGTCGGAAGAAGTAG-3') and AUX1-SacI-R (5’-CTTTACGAGCTCTGTATTCG-3’) generated an *Xba*I-*Sac*I fragment. The primer set AUX1-SacI-F (5’-CGAATACAGAGCTCGTAAAG-3’) and AUX-AflII-R (5’-GCCAAAGCCCTTAAGCAAATG-3’) generated a *Sac*I-*Afl*II fragment. The primer set AUX1-AflII-F (5’-CATTTGCTTAAGGGCTTTGGC-3’) and AUX1-BamHI-R (5’-CACGGATCC GTTCATGGTAA-3’) generated an *Afl*II-*Bam*HI fragment. The resulting 3 fragments were each cloned to pBluescript SK. Each clone was confirmed by sequencing, and the DNA fragments were ligated to generate a full length genomic DNA clone. The resulting genomic *AUX1*was released by *Xba*I and cloned to pART 7, which has the *35S* promoter, released with *Not*I, and cloned to the binary vector pBmart. To clone the genomic *aux1*^{rcr1}, the genomic DNA from the *aux1*^{rcr1} mutant was used as the template for PCR cloning, and the cloning was followed as described for the genomic *AUX1* cloning. To clone genomic *CTR1*, the bacterial artificial chromosome (BAC) clone F17C15 (from ARBC) was restricted with *Xho*I and *Xba*I, and the resulting DNA fragments were sub-cloned to pBluescript SK. The *CTR1* clone was then restricted with *Kpn*I and *Xba*I, and the resulting fragment was cloned to the binary vector pCAMBIA1301. For clones encoding aux1^{rcr1} with the Green Fluorescence Protein (GFP) fusion, the primer AUX1-ATG-KpnI-F (5’-CCAGGTACCATGTCGGAAGGAGTAG-3’) pairing with AUX1-116-EcoRI-R (5’- GAGGAATTCTTTGAAGCTTTTGCCTTC-3’) and AUX1-165-EcoRI-R (5’- GAG GAATTC GTTTATGTAATAAATG-3’) generated *aux1*^{rcr1} DNA fragments encoding aux1$^{rcr1\ 1\text{-}116}$ and aux1$^{rcr1\ 1\text{-}165}$, respectively. The primer set GFP-ATG-EcoRI-F (5’- GCGAATTCATGAGTAAAGGAG-3’) and GFP -BamHI-R (5’- CAGGATCCGATAGATCTGTATAGTTC-3’) was for the GFP-encoding fragment. The primer AUX1-AflII-R (5’-GCCAAAGCCCTTAAGCAAATG-3’) pairing with AUX1-116-BamHI- F (5’-TCGGATCCAACCACGTCATTCAGGTC -3) and AUX1-165-BamHI-F (5’-CA GGATCC GATCATCTGGACAAGAG-3’) generated the aux1$^{rcr1\ 116\text{-}354}$ and aux1$^{rcr1\ 165\text{-}354}$ fragments, respectively. The primer set AUX1-AflII-F (5’-CATTTGCTTAAGGGCTTTGGC-3’) and AUX1-BamHI -R (5’-CACGGATCCGTTCATGGTAA-3’) generated the aux1$^{rcr1\ 354\text{-}485}$ fragment. These *aux1*^{rcr1} fragments and the GFP-encoding fragment were each cloned to pBluescript SK. Each clone was confirmed by sequencing, released by corresponding restriction

enzymes, and sequentially ligated to produce *GFP-116-aux1*^{rcr1} and *GFP-165-aux1*^{rcr1}. The *GFP-116-aux1*^{rcr1} and *GFP-165-aux1*^{rcr1} clones were each restricted by *Kpn*I and *Xba* I, and cloned to pCAMBIA1301 containing the *35S* promoter. Phenotypic analyses of those transformation lines were carried out in the F_3, or higher, generations. The transgenes were each transformed to *Arabidopsis* by *Agrobacterium* as described (Clough and Bent, 1998).

Reference:

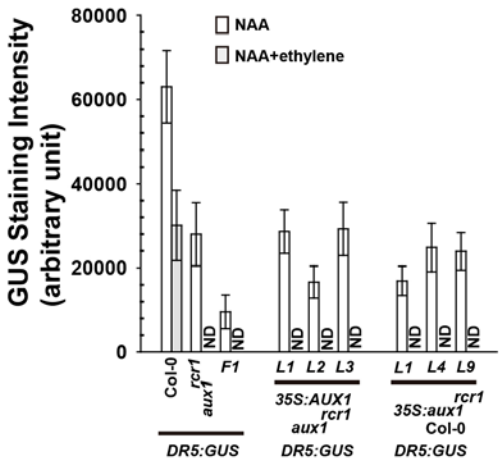
Clough SJ, Bent AF (1998) Floral dip: a simplified method forAgrobacterium-mediated transformation ofArabidopsis thaliana. The Plant Journal **16:** 735-743

Supplemental Figure S2. qRT-PCR of *AUX1* in *aux1*^{rcr1} *35S:AUX1 DR5:GUS* lines.



Supplemental Figure S2. qRT-PCR of *AUX1* in *aux1*^{rcr1} *35S:AUX1 DR5:GUS* lines. *AUX1* and *aux1*^{rcr1} levels in the wild type and *aux1*^{rcr1} are respectively referenced as value of 1 (*AUX1*=1 and *aux1*^{rcr1}=1) for the measurement of *AUX1* levels in *aux1*^{rcr1} *35S:AUX1 DR5:GUS* lines. Data are mean±SE of 3 measurements from 3 independent biological samples.

Supplemental Figure S3. *DR5:GUS* expression in root apexes for genotypes with *aux1*^{rcr1}.



Supplemental Figure S3. *DR5:GUS* expression in root apexes for genotypes with *aux1*^{rcr1}. GUS expression is quantified by measurement of GUS staining intensity with ImageJ (NIH) for at least 15 individual chloral-hydrate-cleared roots (n⩾15) for each genotype in each treatment. Error bars indicate the standard deviation for a mean. F1: the F1 of the cross of the wild type (Col-0) and *aux1*^{rcr1} that carry the *DR5:GUS* transgene. ND: GUS staining is not detectable for NAA and ethylene treatment.