A Mutation in the Anticodon of a Single tRNA$^{\text{ala}}$ Is Sufficient to Confer Auxin Resistance in Arabidopsis[1]

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Auxin-resistant mutants have been useful for dissecting the mechanisms that underlie auxin-mediated biological processes. Here we report the isolation and molecular characterization of a novel auxin-resistant mutant in Arabidopsis (*Arabidopsis thaliana*). Like known mutated AUX/IAA transcription factors, the mutant described here displayed dominant resistance to exogenously supplied auxins (sirtinol, 2,4-dichlorophenoxyacetic acid, indole-3-acetic acid) and a host of pleiotropic phenotypes, including apical hook deformation, defects in lateral root development, reduced stature, and homozygous lethality. This mutant showed the same sensitivity to the ethylene precursor 1-aminocyclopropane carboxylic acid as wild-type plants, and retained the ability to induce *IAA19* expression in response to exogenously supplied indole-3-acetic acid. To our surprise, these phenotypes were not caused by a mutation in an AUX/IAA gene, but rather a mutation in a tRNA$^{\text{ala}}$ gene in which the anticodon was found changed from CGC to CAC. Such a change results in a tRNA that is charged with alanine but recognizes the second most highly used valine codon in Arabidopsis. Therefore, the observed phenotypes are likely the composite of stochastic mutations of many proteins, including downstream effectors.

The plant hormone auxin indole-3-acetic acid (IAA) has been implicated in almost every aspect of plant growth and development ranging from embryogenesis to senescence (for recent reviews on auxin biology and biochemistry, see Dharmasiri and Estelle, 2004; Willemsen and Scheres, 2004; Kepinski and Leyser, 2005a; Woodward and Bartel, 2005). Like any hormone, auxin exerts at least some of its effects by serving as a molecular rheostat for transcriptional regulation, both positive and negative, of a set of target genes. Auxin target genes are canonically defined by the presence of auxin response element (ARE) sequence motifs in their promoter regions, and many have been validated by recent genomic analyses (Ulmasov et al., 1995, 1997; Hagen and Guilfoyle, 2002; Goda et al., 2004; Liu et al., 2005). AREs recruit auxin response factor (ARF) homodimers that directly activate or repress transcription of the proximal gene (Ulmasov et al., 1995, 1997; Abel et al., 1996). Molecular interpretation of the auxin signal is a complex and incompletely defined process; however, the best models based on current data suggest that either (1) in a basal state, ARFs are sequestered as heterodimers by inhibitory subunits (AUX/IAA proteins) that are targeted for degradation in the presence of an auxin signal, liberating the ARFs to homodimerize, bind AREs, and in turn regulate gene expression (Hellman and Estelle, 2002), or (2) that ARFs are constitutively bound to AREs and recruit AUX/IAA proteins when auxin levels are low, repressing the normal function of the ARF (Tiwari et al., 2003). Regardless, degradation of AUX/IAA proteins in response to the auxin signal is a key step in signal propagation that has recently been shown to proceed by ternary complex formation between IAA, a given AUX/IAA protein, and the F-box protein TIR1 (transport inhibitor resistance 1; Dharmasiri et al., 2005; Kepinski and Leyser, 2005b).

Forward genetic screens for auxin-resistant mutants have identified a number of components in the IAA-signaling network including protein ubiquitin-labeling machinery, the dual-specificity phosphatase IBR5 (indole-3-butyric acid response 5), and an assortment of AUX/IAA proteins (for examples, see Leyser et al., 1993; Nagpal et al., 2000; Gray et al., 2001; Schwechheimer et al., 2001; Hellmann et al., 2003; Monroe-Augustus et al., 2003). Most of these screens are designed so as to select for mutants that are resistant to exogenously supplied auxin (usually a synthetic analog of IAA). That is, primary root growth persists when seeds are germinated and grown under cycling white light for several days on plates containing auxin, typically 100 nM of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D). Any mutations that disrupt the uptake (or promote the export), transport, processing, or perception of the auxin are expected to confer auxin resistance. In addition, mutations in genes encoding for downstream effectors that participate in long-range network feedback

mechanisms could also potentially confer auxin resistance, but to date none have been reported with the possible exception of the dual-specificity phosphatase IBR5, whose function has yet to be fully elucidated (Monroe-Augustus et al., 2003).

Here we report the phenotypic characterization and cloning of a novel auxin-resistant mutant in Arabidopsis (*Arabidopsis thaliana*). Like the phenotypes caused by the reported mutations in AUX/IAA genes, the phenotypes associated with the mutant described here segregate in dominant fashion and include resistance to several auxins (IAA, 2,4-D, and sirtinol), apical hook deformation, reduced stature, and defects in lateral root development. Similar to *axr5/iaa1* plants (Yang et al., 2004), the rosette leaves of this mutant were more rounded with shortened petioles, but it is distinct in that it appears to retain sensitivity to ethylene and the mutation is homozygous lethal. Surprisingly, positional cloning on the basis of 2,4-D resistance revealed that the mutation linked to the observed phenotypes was not found in an AUX/IAA gene, but in the anticodon of a tRNA^{ala}. The mutation transforms this tRNA^{ala} into a tRNA that remains charged with Ala but now recognizes a Val codon (GUG), which by codon bias analysis is found to be the second most highly utilized Val codon in Arabidopsis. The presumed molecular effect of this mutation is that some population of Vals in the proteome encoded by GUG will be replaced by Ala in a stochastic way, and that the bulk composite confers auxin resistance and other auxin-related phenotypes. Yanagida and colleagues have recently recovered similar tRNA^{ala} anticodon mutations from *Schizosaccharomyces pombe* while screening for suppressors of mutations in the Cdc16 subunit of the anaphase-promoting complex (Kimata and Yanagida, 2004). These mutants, while suppressing the anaphase-promoting complex phenotype (arrest at the metaphase to anaphase transition), conferred dominantly segregating phenotypes at anaphase spindle elongation that were lethal at restrictive temperatures because of chromosome missegregation at cell division (Kimata and Yanagida, 2004).

RESULTS

Identification of a Dominant Auxin-Resistant Mutant That Is Homozygous Lethal

We screened the M2 population of ethyl methanesulfonate-mutagenized Columbia (Col) seeds for plants that were resistant to 20 μM sirtinol to identify auxin-resistant mutants (Zhao et al., 2003; Cheng et al., 2004; Dai et al., 2005). When the mutant described here was backcrossed to wild-type Col or outcrossed to Landsberg *erecta*, the resulting F$_1$ populations of plants were resistant to both sirtinol and 2,4-D, indicating that the auxin resistance segregates in dominant fashion. However, we later found that this mutant was originally isolated in the presence of background mu-

tations as successive backcrosses reduced the number of homozygous progeny recovered from self-fertilizing heterozygous plants. Ultimately, the homozygous mutant was determined to be embryonic lethal.

Phenotypic Analyses

All analyses/comparisons were performed with heterozygous mutant plants, which were confirmed by genotyping (below). Three-day-old light-grown mutant and wild-type seedlings are compared in Figure 1, A to C. At this stage, the difference between wild-type and heterozygous mutant plants is subtle when grown on unsupplemented Murashige and Skoog (MS) medium and consists only of a more flattened architecture of the cotyledons in the mutant, so that they are perpendicular to the hypocotyl (Fig. 1A). Though subtle, genotyping progeny of self-fertilized heterozygous plants grown on unsupplemented MS and scored on this basis confirmed that this trait was linked to the mutation (72/72). Resistance to sirtinol, 2,4-D, and IAA, as judged by primary root elongation in the presence of typically inhibiting concentrations of these compounds, is also readily discerned in the mutant at this stage (Fig. 1, B, C, and K). Finally, after 7 d on unsupplemented MS, mutant seedlings were found to have a defect in lateral root development (Fig. 1D).

In the dark, the mutant lacked the normal apical hook structure and was found to be resistant to sirtinol and 2,4-D in both the root and hypocotyl tissues (Fig. 1, E–G). Somewhat surprisingly, the mutant appears to retain full sensitivity to the ethylene precursor 1-aminocyclopropane-1-carboxlyic acid (ACC; Fig. 1, H and L).

As the plants mature, more phenotypes are evident in the mutant. The rosette leaves are more rounded and the petioles are shorter in mutant and recapitulation line (20 lines generated, below) plants (Fig. 1I), and all lines are reduced in stature relative to wild type (Fig. 1J).

Molecular Cloning and Recapitulation

Linkage analysis placed the mutated gene in the middle of the bottom arm of chromosome IV, and fine mapping narrowed the interval to an 80-kb region between markers F9D16-98 and T32A16-75 (Fig. 2A). DNA sequencing of all open reading frames in this region identified a single G-to-A transition in the anticodon (CGC to CAC) of a tRNA^{ala} encoded by the gene At4g23915. This change results in a tRNA that is charged with Ala but expected to recognize a codon for Val (GUG). It should be noted that unlike other tRNAs, which have multiple determinants located in the arms and core of the structure, sometimes including the anticodon, the indicated (*) G3-U70 wobble conserved among tRNA^{ala}s (Fig. 2B) is the only major identity determinant for tRNA^{ala} identity (Hou and Schimmel, 1988; McClain and Foss, 1988). Therefore,
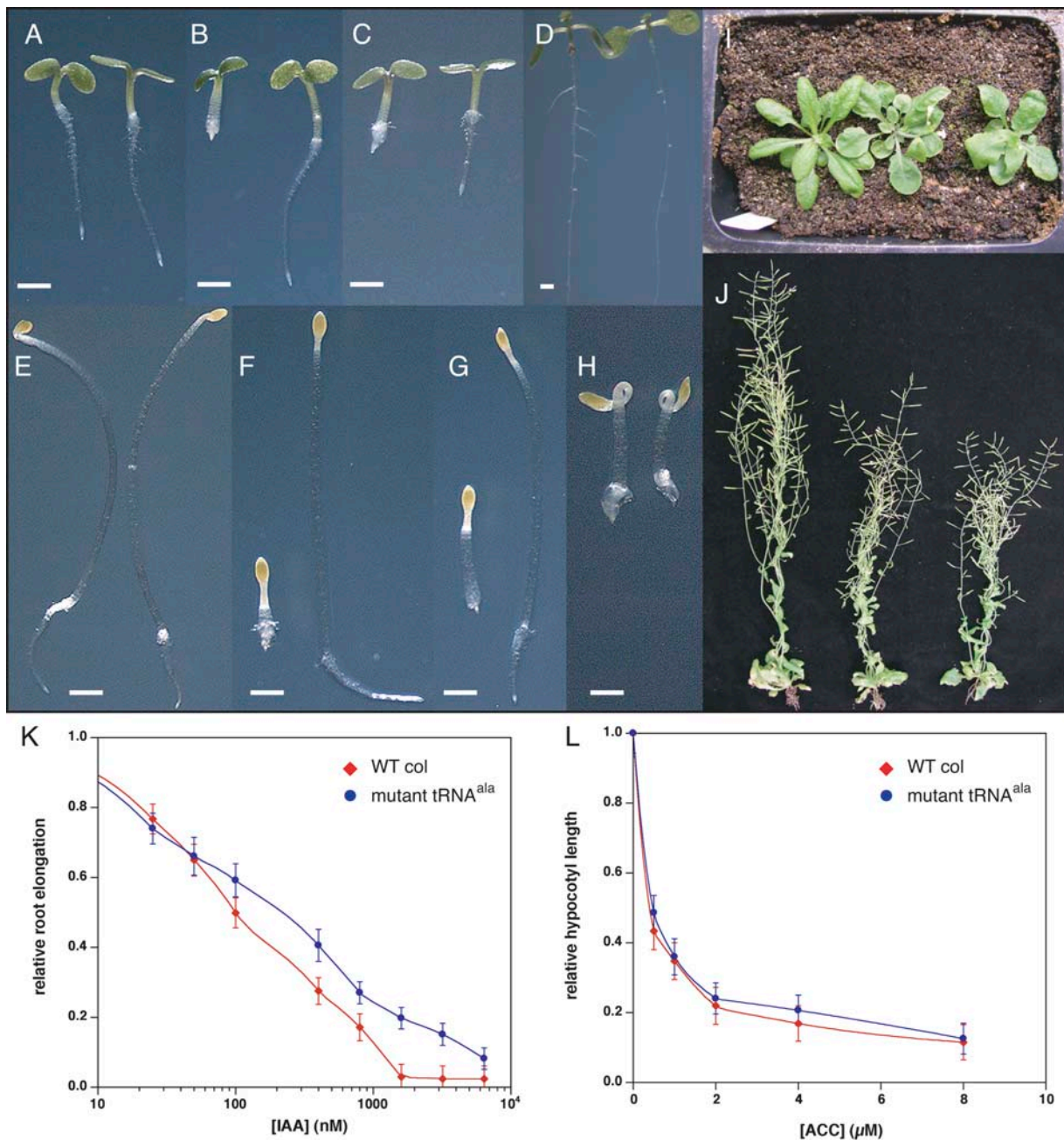
Figure 1. Phenotypic analysis of an auxin-resistant tRNA[ala] anticodon mutant from Arabidopsis. Seedling phenotypes under the given conditions are shown in sections A to H, wild type on the left, heterozygous mutant on the right. A to C, Three-day-old light-grown seedlings on 0.5× MS (A), 0.5× MS supplemented with 10 μM of sirtinol (B), or 100 nM of the synthetic auxin 2,4-D (C). D, Seven-day-old light-grown seedlings on 0.5× MS. E to H, Three-day-old dark-grown seedlings on 0.5× MS (E), 0.5× MS supplemented with 2 μM sirtinol (F), 250 nM 2,4-D, or 2 μM of the ethylene precursor ACC (G). Later stage phenotypes are shown in I and J, wild type on the left, heterozygous mutant in the center, and heterozygous recapitulation line on the right. I, Fully formed rosette leaf stage. J, Mature plants. Hormone response curves for IAA and ACC are provided in sections K and L, respectively.

we only expect to be able to obtain similar results with mutations in other tRNA[ala]s. However, as a caveat, previous studies have focused mostly on *Escherichia coli*, *Saccharomyces cerevisiae*, and *Thermus thermophilus*, which differ significantly from one another at the level of tRNA identity determinacy, and, therefore, it is

conceivable that other, non-Ala-charging, tRNA mutants could be recovered from genetic screens in Arabidopsis (for review, see Giegé et al., 1998).

To confirm that this mutation was responsible for the observed phenotypes, we transformed wild-type Col plants with a construct harboring the mutated
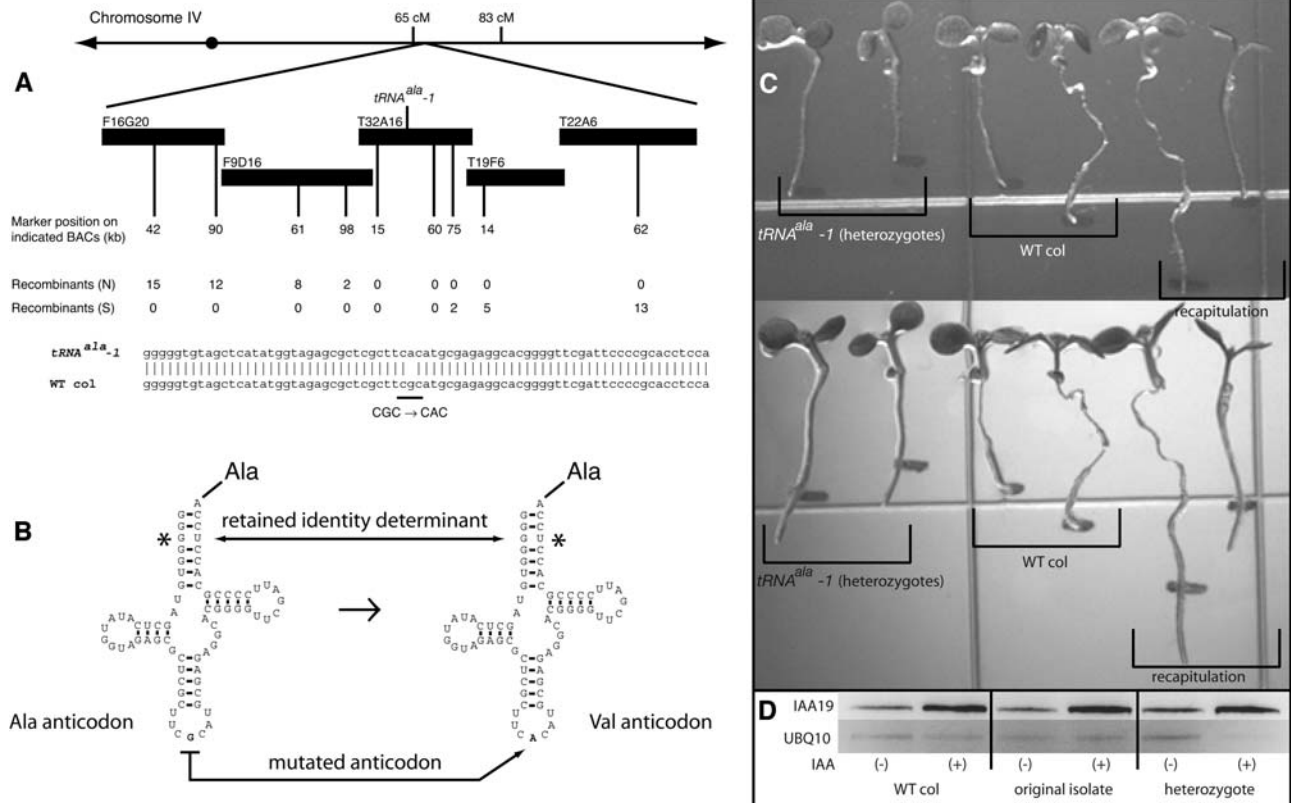
Figure 2. Cloning of the mutant tRNA^{ala} and recapitulation of auxin resistance. A, Mapping data. BAC, Bacterial artificial chromosome; cM, centimorgan, the unit of genetic distance describing the recombination frequency between two loci as 1%/cM. The observed G-to-A transition in the tRNA gene is highlighted. B, Unlike other tRNAs, which have multiple determinants located in the arms and core of the structure, the indicated (*) G3-U70 wobble conserved among tRNA^{ala}s is the only major identity determinant (Hou and Schimmel, 1988; McClain and Foss, 1988). Therefore, we expect the documented phenotypes to occur only with this or other mutated tRNA^{ala}s. C, Recapitulation. The top section is a picture of seedlings (right two, tRNA^{ala} mutant heterozygotes; center two, wild type; left two, recapitulation lines [text]) immediately after transfer to 0.5 × MS vertical plates supplemented with 100 nM 2,4-D; the bottom section shows the same seedlings after 24 h continued growth. D, *IAA19* induction. RT-PCR shows that *IAA19* expression is induced by IAA (10 μM) in the mutant tRNA^{ala} lines. *UBQ10* expression is not regulated by IAA and is included as a control.

tRNA^{ala} under the transcriptional control of its natural promoter. This was sufficient to both confer auxin resistance and recapitulate the adult phenotypes (Figs. 1, I and J, and 2C).

Effect of the Mutated tRNA^{ala} on Auxin-Induced Gene Expression

One of the hallmarks of the auxin-mediated transcriptional response is the induction of AUX/IAA genes. *IAA19* expression is robustly induced during the seedling stage (Nakamura et al., 2003), so we asked whether this was compromised in the mutated tRNA^{ala} lines reported here. Using reverse transcription (RT)-PCR, seedlings of both the original isolate (which was homozygous for the mutation) and of the backcrossed heterozygous line showed *IAA19* induction in response to IAA comparable to that observed in wild-type Col seedlings (Fig. 2D). This is minimally consistent with a downstream effect giving rise to the

observed phenotypes, as opposed to a defect at or before transcription.

Arabidopsis tRNA Bioinformatics and Codon Bias Analysis

tRNAscan-SE analysis of the Arabidopsis genome previously indicated the presence of 630 standard tRNAs, no selenocysteine (UCA) or suppressor (CUA, UUA) tRNAs, one tRNA of unknown isotype, and eight predicted pseudogenes (http://lowelab.ucsc.edu/GtRNAdb/Athal/; Lowe and Eddy, 1997). Of those, 33 recognize a codon for Ala (using CGC [7], UGC [10], and AGC/IGC [16] anticodons) and 30 recognize a codon for Val (using CAC [8], UAC [7], and AAC/IAC [15] anticodons). Their genomic locations and anticodon and codon recognition distribution patterns are shown in Figure 3A. Using a set of genes that encode for large proteins with high Ala and Val content (BIG [gb|AF507018], ATM [ref|NM_114689], TOR [ref|NM_103891], and myosin

A

Arabidopsis tRNA^{ala} genes

chromosomal address	BAC	anticodon	codon recognized
At1g64420	F15H21/F1N19	CGC	GCG
At2g22580	T9I22/F14M13	CGC	GCG
At3g61755	F21F14/F15G16	CGC	GCG
● At4g23915	T32A16	CGC	GCG
At4g27885	T27E11	CGC	GCG
At5g08075	T22D6	CGC	GCG
At5g60963	MSL3	CGC	GCG
At1g08870	F7G19	UGC	GCA
At1g13840	F16A14	UGC	GCA
At1g13850	F16A14	UGC	GCA
At1g26490	T1K7	UGC	GCA
At1g54670	T22H22	UGC	GCA
At2g03490	T4M8	UGC	GCA
At2g47100	F14M4	UGC	GCA
At3g62245	T17J13	UGC	GCA
At3g63003	T20O10	UGC	GCA
At3g63006	T20O10	UGC	GCA
At1g06610	F12K11	AGC/IGC	GCU/GCC/GCA
At1g07580	F22G5	AGC/IGC	GCU/GCC/GCA
At2g02600	T8K22	AGC/IGC	GCU/GCC/GCA
At2g02900	T17M13	AGC/IGC	GCU/GCC/GCA
At2g30850	F7F1	AGC/IGC	GCU/GCC/GCA
At3g28685	MZN14	AGC/IGC	GCU/GCC/GCA
At3g28695	MZN14	AGC/IGC	GCU/GCC/GCA
At4g11355	F8L21	AGC/IGC	GCU/GCC/GCA
At4g12065	F16J13	AGC/IGC	GCU/GCC/GCA
At4g26675	T15N24/F10M23	AGC/IGC	GCU/GCC/GCA
At5g01365	T10O8	AGC/IGC	GCU/GCC/GCA
At5g07675	MBK20	AGC/IGC	GCU/GCC/GCA
At5g39895	MYH19	AGC/IGC	GCU/GCC/GCA
At5g43455	MWF20	AGC/IGC	GCU/GCC/GCA
At5g55505	MTE17	AGC/IGC	GCU/GCC/GCA
At5g61445	MFB13	AGC/IGC	GCU/GCC/GCA

Arabidopsis tRNA^{val} genes

chromosomal address	BAC	anticodon	codon recognized
At2g22110	T16B14	CAC	GUG
At3g11395	F24K9	CAC	GUG
At3g26747	MDJ14	CAC	GUG
At4g39672	F23K16/T19P19	CAC	GUG
At5g05985	K18J17	CAC	GUG
At5g15175	F8M21	CAC	GUG
At5g38155	MXA21	CAC	GUG
At5g56975	MHM17	CAC	GUG
At1g01870	F22M8	UAC	GUA
At2g03790	F19B11	UAC	GUA
At2g29520	F16P2	UAC	GUA
At3g07025	F17A9	UAC	GUA
At3g50835	F18B3	UAC	GUA
At4g16235	FCA5	UAC	GUA
At5g44375	K9L2	UAC	GUA
At1g12510	F5O11/T12C24	AAC/IAC	GUU/GUC/GUA
At1g17670	F11A6	AAC/IAC	GUU/GUC/GUA
At1g24080	T23E23	AAC/IAC	GUU/GUC/GUA
At1g30840	T17H7	AAC/IAC	GUU/GUC/GUA
At3g45935	F16L2	AAC/IAC	GUU/GUC/GUA
At3g45955	F16L2	AAC/IAC	GUU/GUC/GUA
At3g45965	F16L2	AAC/IAC	GUU/GUC/GUA
At3g49925	T16K5/F3A4	AAC/IAC	GUU/GUC/GUA
At3g59923	F24G16	AAC/IAC	GUU/GUC/GUA
At4g01355	F2N1	AAC/IAC	GUU/GUC/GUA
At4g11985	F16J13	AAC/IAC	GUU/GUC/GUA
At5g02435	T22P11/T1E22	AAC/IAC	GUU/GUC/GUA
At5g09655	F17I14/MTH16	AAC/IAC	GUU/GUC/GUA
At5g59945	MMN10	AAC/IAC	GUU/GUC/GUA
At5g64505	MUB3	AAC/IAC	GUU/GUC/GUA

B

	Alanine				Valine			
mRNA codon	GCG	GCA	GCU	GCC	GUG	GUA	GUU	GUC
BIG	27	113	128	42	103	52	134	66
ATM	17	69	79	19	52	37	93	37
TOR	14	76	94	33	43	30	92	25
Myosin	4	34	33	4	21	9	37	6
total	62	292	334	98	219	128	356	134
codon bias	0.079	0.372	0.425	0.125	0.262	0.153	0.425	0.160

Figure 3. Informatic analysis of tRNA^{ala} and tRNA^{val} and codon usage bias in Arabidopsis. A, The tRNA^{ala} and tRNA^{val} genes in Arabidopsis. Note that there are three types of tRNAs encoded to recognize four types of codons in each instance. The gene cloned in this study is highlighted and indicated (●). B, Codon usage bias analysis. A total of 786 Ala codons and 837 Val codons were analyzed.

[ref|NM112886]; 786 total Ala codons, 837 total Val codons), we estimated the codon bias in Arabidopsis. There appears to be a very strong bias for certain Ala codons (GCU>GCA≫GCC>GCG), and a weaker but still easily measured bias in Val codon usage (GUU>GUG>GUA≈GUC; Fig. 3B). In the mutant described here, a tRNA^{ala} that recognizes the codon GCG is transformed into a tRNA that is charged with Ala but that recognizes the Val codon GUG; therefore, a tRNA that is presumably used only very sparingly (14 copies are encoded to recognize approximately 8% of Ala codons) is converted to a tRNA that could be used more frequently (16 copies of the CAC anticodon tRNA for Val are encoded to recognize approximately 26% of Val codons), and serves as a mutator. Interestingly, only one copy of the mutated tRNA^{ala} in the presence of 16 copies of GUG recognizing wild-type tRNA^{val} is sufficient to give rise to the phenotypes documented here, and two copies are lethal. As a caveat, it is not trivial to determine the expression pattern of the various tRNAs (because many of the different genes have identical nucleotide sequences, and their inherent hairpin structures are not amenable to RT-PCR) and to know whether they all contribute equally to the pool.

DISCUSSION

Here we have shown that a mutation in the anticodon of a tRNA^{ala} can in many ways mimic the phenotypes observed in several reported AUX/IAA gene mutants. In both cases, mutant phenotypes are dominant and include resistance to exogenously supplied auxin, reduced stature, and defects in lateral root development. The phenotypes associated with the mutant tRNA reported here are perhaps most similar to those recently reported with *axr5/iaa1*, which also include more rounded rosette leaves and shortened petioles (Yang et al., 2004).

The precise reasons for the similar phenotypes is not clear at present; however, in both cases (the mutant tRNA versus AUX/IAA mutants) the observed phenotypes are likely to be composites of downstream effects. With the mutant tRNA, we expect that a steady-state level of many mutated proteins contributes to the phenotypes, though a more direct effect or a major effect on one or more specific components cannot be ruled out. For example, 15 different AUX/IAA proteins contain at least one Val residue encoded by GTG within their respective domain II segments, the genetic region where all recovered AUX/IAA mutations have occurred. Therefore, Val to Ala substitutions in AUX/IAA proteins could contribute to auxin resistance, but at this time it is not clear whether that is a major determinant because (1) none of these Val residues are invariant among AUX/IAA proteins in Arabidopsis, and (2) mapped mutations have occurred only in DNA sequences encoding either a Gly residue or one of two Pro in that region. With AUX/IAA mutants, many

genes are expected to be misregulated with respect to the auxin signal, and it is not known exactly how the observed pleiotropic phenotypes arise. However, given the apparent sensitivity to ACC and the potent IAA-mediated induction of *IAA19*, it seems likely that the mutated tRNA exerts its greatest influence over proteins encoded by downstream effector genes of the auxin pathway rather than previously identified components such as those that comprise protein degradation machinery (all of which exhibit resistance to ACC) and thus may be a useful tool for studying these auxin regulated pathways.

One such pathway is auxin-regulated cell division. It has recently been reported that transgenic Arabidopsis lines carrying a Ran-binding protein 1c RNAi knockdown construct were defective in lateral root development but auxin hypersensitive, suggesting that Ran-binding protein 1c functions to suppress certain auxin effects yet regulates mitosis in root tips (Kim et al., 2001). Given that many mutations to emerge from genetic screens, including the mutated tRNA described here, are defective in lateral root development but auxin resistant, the interface of auxin signaling and mitosis is expected to be complex. However, recent experiments in *S. pombe* have shown that tRNA^{ala} anticodon mutations can confer specific dominant defects in late mitosis due to abnormal spindle dynamics in that organism (Kimata and Yanagida, 2004). Therefore, the mutant described here might be able to be used for studying the auxin-signaling/mitosis interface, and it provides a handle to leverage information regarding translation quality control networks in Arabidopsis, a virtually unexplored avenue for research.

MATERIALS AND METHODS

Mutagenesis and the Sirtinol Resistance Screen

Ethylmethane sulfonate-mutagenized Arabidopsis (*Arabidopsis thaliana*) Col M2 seeds were purchased from Lehle Seeds. Genetic screens for sirtinol-resistant mutants were performed as previously described (Zhao et al., 2003; Cheng et al., 2004; Dai et al., 2005). In brief, M2 seeds were germinated on 0.5× MS/agar plates supplemented with 20 μM sirtinol and allowed to grow under cycling white light (16 h light/8 h darkness) for 6 d, after which time seedlings were scored for resistance to sirtinol on the basis of primary root elongation, then transplanted directly to soil to set seeds.

Positional Cloning and Recapitulation

The mutant tRNA^{ala} described here was cloned by a map-based strategy (Lukowitz et al., 2000) on the basis of its resistance to the synthetic auxin 2,4-D. Polymorphic sequence markers were designed with reference to the polymorphisms between the Col and Landsberg ecotypes as defined by Monsanto (accessed by http://www.arabidopsis.org). The observed phenotypes were recapitulated by cloning a genomic fragment containing the mutated tRNA and its promoter elements into the binary vector pZP211, and then transforming that construct to wild-type Arabidopsis (Col) plants. The primers used to amplify the approximately 1.0-kb fragment were 5′-GGGGTACCA-CAAAGCTTTTACGGGGTTGTTCCAGTTT-3′ and 5′-TTTCTGCAGTTTTAC-GTCAAATTCTTTAATTCACTT-3′. Seeds from transformed plants were sown on 0.5× MS containing 30 μg/mL kanamycin, stratified for 2 d at 4°C, then allowed to germinate and grow under cycling white light for 6 d. Those plants that were resistant to kanamycin were transferred to vertical MS plates

supplemented with 100 nM 2,4-D and allowed to grow for an additional 24 h. Root elongation was observed in all plants containing the kanamycin-resistant transgene.

Hormone Response Assays

For auxin, 5-d-old seedlings were transferred from 0.5× MS plates to vertical 0.5× MS plates supplemented with varying concentrations of IAA. The primary root tips were marked, and then seedlings were allowed to grow under cycling white light for an additional 2 d, after which continued root elongation was quantified using the public domain NIH Image program (developed at the United States National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). All data points are derived from a minimum of 10 plants.

For ethylene, seeds were sown on 0.5× MS plates containing varying concentrations of the ethylene precursor ACC. These seeds were stratified for 2 d at 4°C, germinated under white light for exactly 2 h, and then the plates were wrapped in foil for exactly 72 h, after which time hypocotyl length was quantified with NIH Image. All data points are derived from a minimum of 15 plants.

IAA19 Induction

Five-day-old seedlings grown on 0.5× MS plates were immersed in 0.5× MS liquid medium supplemented with IAA to a final concentration of 10 μM for 2 h. Seedlings were then harvested and total RNA was prepared using the Rneasy Plant mini kit (Qiagen) following the instructions provided by the manufacturer. Following first-strand cDNA synthesis, transcripts from *IAA19* and *UBQ10* were analyzed by PCR using the following primer combinations: *IAA19*, 5'-TGGTGACAACTGCGAATACG-3' and 5'-TCACTCGTCTACTCC-TCTAG-3'; and *UBQ10*, 5'-GTCCTCAGGCTCCGTGGTG-3' and 5'-TGCCA-TCCTCCAACTGCTTTC-3'.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number At4g23915.

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