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Article Addendum

Ethylene-Stimulated Nutations Do Not Require ETR1 Receptor Histidine Kinase Activity

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Addendum to:

Ethylene Stimulates Nutations That Are Dependent on the ETR1 Receptor

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ABSTRACT

Ethylene influences the growth and development of plants through the action of receptors that have homology to bacterial two-component receptors. In bacteria these receptors function via autophosphorylation of a His residue in the kinase domain followed by phosphotransfer to a conserved Asp residue in a response regulator protein. In *Arabidopsis*, two of the five receptor isoforms are capable of His kinase activity. However, the role of His kinase activity and phosphotransfer is unclear in ethylene signaling. A previous study showed that ethylene stimulates nutations of the hypocotyl in etiolated *Arabidopsis* seedlings that are dependent on the ETR1 receptor isoform. The ETR1 receptor is the only isoform in *Arabidopsis* that contains both a functional His kinase domain and a receiver domain for phosphotransfer. Therefore, we examined the role that ETR1 His kinase activity and phosphotransfer plays in ethylene-stimulated nutations.

The gaseous plant hormone ethylene has a role in a variety of physiological events in higher plants such as seed germination, abscission, senescence, fruit ripening, and growth regulation.[1] In etiolated *Arabidopsis* seedlings, ethylene causes reduced growth of the hypocotyl and root, increased radial expansion of the hypocotyl, and increased tightening of the apical hook.[2,3]

Previous studies have identified components in the ethylene signaling pathway and led to an inverse-agonist model for signal transduction.[4,5] According to this model, responses to ethylene are mediated by a family of five receptors (ETR1, ERS1, ETR2, EIN4, ERS2) in *Arabidopsis* that have homology to bacterial two-component receptors.[6-9] In bacterial systems, two-component receptors transduce signal via the autophosphorylation of a His residue in the kinase domain, followed by the transfer of phosphate to a conserved Asp residue in the receiver domain of a response regulator protein.[10] The ethylene receptors of plants can be divided into two subfamilies based on sequence homology in the ethylene-binding domains.[11] ETR1 and ERS1 belong to subfamily I, contain all amino acid residues needed for His kinase activity,[6,12] and show His kinase activity in vitro.[13,14] ETR2, EIN4, and ERS2 belong to subfamily II, contain degenerate His kinase domains[7,9] and have Ser/Thr kinase activity in vitro.[14] ERS1 shows both His and Ser/Thr kinase activities in vitro depending on the assay conditions used.[14] While the kinase domain of ETR1 appears to be required for signaling,[15] kinase activity is not.[15-17] It is unclear whether or not histidine kinase activity is involved in ethylene signaling, although, this activity might be involved in growth recovery after ethylene removal.[17]

Recently, high-resolution, time-lapse imaging revealed that prolonged treatment with ethylene stimulates nutational bending of etiolated *Arabidopsis* hypocotyls.[18] Nutations are oscillatory bending movements caused by localized differential growth[19] that were originally termed "circumnutations".[20] Nutations have been posited to be important for seedlings to penetrate through the soil[20] and thus could be critical for seedling survival. In support of this hypothesis, nutations of rice roots have been reported to increase soil penetration.[21]

Mutational analysis revealed that many of the known ethylene signaling components including CTR1, EIN2, EIN3 and EIL1 are involved in signaling leading to ethylene-stimulated nutations.[18] Surprisingly, loss-of-function mutations in *ETR1* eliminated ethylene-stimulated nutations while combinatorial loss-of-function mutations in the other four receptor isoforms led to constitutive nutations in air.[18] These results support a model where all the receptors are involved in ethylene-stimulated nutations but the ETR1 receptor is required for and has a contrasting role from the other receptor isoforms in this nutation phenotype. Since the ETR1 receptor is the only receptor isoform that contains both a functional His-kinase domain and a receiver domain,[6,13,14] the roles of His kinase

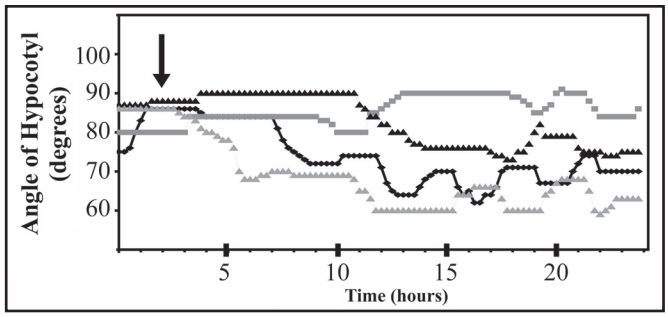

Figure 1. Ethylene stimulates nutations of *etr1-7* seedlings transformed with a kinase-inactivated *ETR1* transgene. The hypocotyl angles for four *etr1-7* mutants transformed with a kinase-inactivated genomic *ETR1* transgene (*gETR1*(G_2)) are shown. Transformants were obtained from Eric Schaller and have been described previously.[22] In this and the following figure, etiolated *Arabidopsis* seedlings were imaged from the side at 15 min intervals while growing along a vertically orientated agar plate and the hypocotyl angle measured as described previously.[18] Black and gray lines are used to help distinguish the movements of individual seedlings. All seedlings were grown in the presence of 5 μM AVG to block biosynthesis of ethylene by the seedlings. Seedlings were grown in air for 2 h prior to treatment with 10 μL L^{-1} ethylene (ℰ).

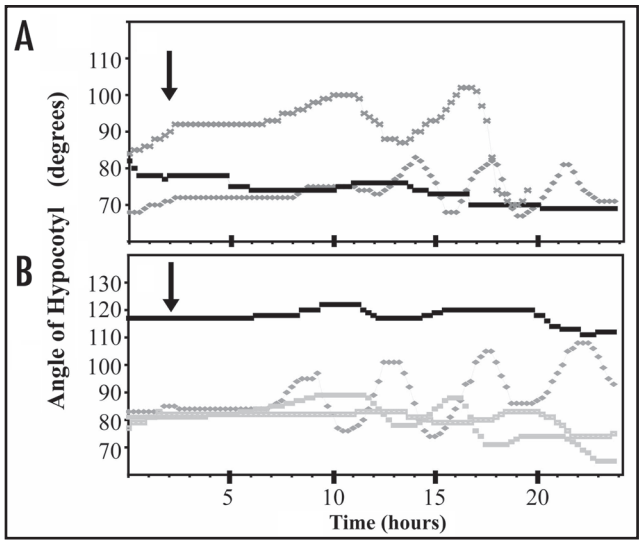

Figure 2. Ethylene stimulates nutations of *etr1-6 etr2-3 ein4-4* seedlings transformed with an *ETR1* transgene mutated at Asp_{659}. The hypocotyl angles from seven *etr1-6 etr2-3 ein4-4* triple mutants transformed with an *ETR1* transgene mutated at Asp_{659} *(getr1[D])* are shown in two panels. One seedling in (A) (black) had no measurable nutations while one in (B) (black) had very small nutations.

activity and phosphorelay in the nutation phenotype were examined in the current study.

Previous work showed that the nutation phenotype in *etr1-7* loss-of-function mutants could be rescued with a wild-type, genomic *ETR1* transgene.[18] *Etr1-7* mutants transformed with a kinase-inactivated genomic *ETR1* transgene (*gETR1* (G_2)) where the two conserved glycines in the G_2 box of the histidine kinase domain (G545, G547) were changed to alanines were examined to determine if ETR1 His kinase activity is required for ethylene-stimulated nutations. This construct lacks histidine autophosphorylation in vitro.[22] Figure 1 shows that ethylene stimulates nutations in *etr1-7 gETR1*(G_2) seedlings. The period of these nutations was 4.7 ± 1.5 h which is similar to values obtained previously for wild-type seedlings (4.7 ± 1h) and somewhat longer than *etr1-7* seedlings transformed with wild-type, genomic *ETR1* (3.2 ± 0.6 h). However, the amplitude of these nutations (3.7 ± 1.0°) was approximately half that of nutations previously observed in wild-type seedlings (9.1 ± 6.0°) as well as *etr1-7* seedlings transformed with wild-type, genomic *ETR1* (8.2 ± 3.6°). This suggests that ETR1 histidine kinase activity is not required for ethylene-stimulated nutations but might have a role in modulating nutation amplitudes.

To determine whether phosphotransfer through the receiver domain of ETR1 is required for the nutation phenotype, seedlings deficient in ethylene receptor isoforms containing a receiver domain (ETR1, ETR2, EIN4) were transformed with a mutant *ETR1* transgene lacking the conserved Asp_{659} required for phosphotransfer (*getr1-[D]).* Previous work showed that *etr1-6 etr2-3 ein4-4* triple loss-of-function mutant seedlings failed to nutate and this nutation phenotype could be rescued when these mutants were transformed with wild-type, genomic *ETR1* transgene.[18] Similarly, transformation of the *etr1-6 etr2-3 ein4-4* triple mutants with *getr1-[D]* rescued the nutation phenotype in most seedlings observed (Fig. 2). However, some seedlings (four of the eleven observed) failed to nutate. The reason for this variable rescue is unclear but could reflect differences in expression levels of the mutant transgene in individual plants. Alternatively, this variable rescue could reflect functional differences between the mutant and wild-type transgene suggesting a modulating role for phosphotransfer through the receiver domain of ETR1. Two independent lines were observed with similar results. Of those that did nutate, the period of nutations was 5.0 ± 1.2 h and the amplitude 7.6 ± 3.8° which is similar to values obtained previously for wild-type plants as well as plants transformed with a wild-type, genomic *ETR1* transgene.[18]

Conclusions from this and the previous study are that the ETR1 receptor has a unique role in ethylene-stimulated nutations. However, this role does not require either histidine kinase activity or phosphotransfer through the receiver domain of ETR1.

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