January 2006

Ethylene Stimulates Nutations That Are Dependent on the ETR1 Receptor

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Ethylene Stimulates Nutations That Are Dependent on the ETR1 Receptor[1][W][OA]

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Ethylene influences a number of processes in Arabidopsis (*Arabidopsis thaliana*) through the action of five receptors. In this study, we used high-resolution, time-lapse imaging to examine the long-term effects of ethylene on growing, etiolated Arabidopsis seedlings. These measurements revealed that ethylene stimulates nutations of the hypocotyls with an average delay in onset of over 6 h. The nutation response was constitutive in *ctr1-2* mutants maintained in air, whereas *ein2-1* mutants failed to nutate when treated with ethylene. Ethylene-stimulated nutations were also eliminated in *etr1-7* loss-of-function mutants. Transformation of the *etr1-7* mutant with a wild-type genomic *ETR1* transgene rescued the nutation phenotype, further supporting a requirement for ETR1. Loss-of-function mutations in the other receptor isoforms had no effect on ethylene-stimulated nutations. However, the double *ers1-2 ers2-3* and triple *etr2-3 ers2-3 ein4-4* loss-of-function mutants constitutively nutated in air. These results support a model where all the receptors are involved in ethylene-stimulated nutations, but the ETR1 receptor is required and has a contrasting role from the other receptor isoforms in this nutation phenotype. Naphthylphthalamic acid eliminated ethylene-stimulated nutations but had no effect on growth inhibition caused by ethylene, pointing to a role for auxin transport in the nutation phenotype.

The gaseous plant hormone ethylene influences a number of processes in higher plants, such as seed germination, abscission, senescence, fruit ripening, and growth regulation. In etiolated seedlings, ethylene causes a number of changes, including reduced growth of the hypocotyl and root, increased radial expansion of the hypocotyl, altered geotropism, and increased tightening of the apical hook (Abeles et al., 1992). Biochemical and mutational studies have identified many components in the ethylene-signaling pathway and led to an increasingly refined model for signal transduction (Guo and Ecker, 2004; Chen et al., 2005).

According to this model, responses to ethylene are mediated by a family of five receptors (ETR1, ERS1, ETR2, EIN4, ERS2) in Arabidopsis (*Arabidopsis thaliana*) that have homology to bacterial two-component receptors (Chang et al., 1993; Hua et al., 1998; Hua and Meyerowitz, 1998; Sakai et al., 1998). In bacterial systems, two-component receptors transduce signal via autophosphorylation of a His residue in the kinase domain, followed by transfer of phosphate to a conserved Asp residue in the receiver domain of a response regulator protein (West and Stock, 2001). Ethylene receptors can be divided into two subfamilies. Subfamily I consists of ETR1 and ERS1, which contain all amino acid residues needed for His kinase activity (Chang et al., 1993; Hua et al., 1995) and show His kinase activity in vitro (Gamble et al., 1998; Moussatche and Klee, 2004). Subfamily II includes ETR2, EIN4, and ERS2, which contain degenerate His kinase domains (Hua et al., 1998; Sakai et al., 1998) and have Ser/Thr kinase activity in vitro. ERS1 is capable of both His and Ser/Thr kinase activity in vitro, depending on the assay conditions used, and is most likely a Ser/Thr kinase in vivo (Moussatche and Klee, 2004). Whereas the kinase domain of ETR1 appears to be required for signaling (Qu and Schaller, 2004), kinase activity per se does not (Wang et al., 2003; Binder et al., 2004b; Qu and Schaller, 2004). It is unclear what, if any, role His kinase activity plays in ethylene signaling. It is possible that the His kinase activity of ETR1 is not involved with ethylene signaling but rather some other function, such as growth recovery after ethylene removal (Binder et al., 2004b).

Ethylene receptors are believed to transduce signal via Ser/Thr kinase activity in CTR1, which has homology to *Raf* mitogen-activated protein kinase kinase kinases (Kieber et al., 1993; Huang et al., 2003). Genetic studies indicate that CTR1 negatively regulates the

[1] This work was supported by the National Science Foundation (grant MCB–0131564 to the late Anthony B. Bleecker, who passed away in January 2005).

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www.plantphysiol.org/cgi/doi/10.1104/pp.106.087858

ethylene response pathway by inhibiting activity of EIN2, which is required for responses to ethylene (Alonso et al., 1999). This model posits that ethylene binding to the receptors reduces the activity of the receptors, leading to reduced activity of CTR1 protein and an increase in activity of EIN2 protein along with subsequent signaling associated with it. Two transcription factors, EIN3 and EIL1, act downstream of EIN2 in the ethylene-signaling pathway (Chao et al., 1997; Alonso et al., 2003). EIN3 in turn regulates the expression of other transcription factors, such as ERF1 (Chao et al., 1997; Solano et al., 1998). Several recent reports show that ethylene leads to an increase in EIN3 protein levels (Guo and Ecker, 2003; Yanagisawa et al., 2003; Gagne et al., 2004). In the absence of ethylene, EIN3 is rapidly degraded by the ubiquitin/26S proteasome pathway using an SCF E3 complex containing the EBF1 and EFB2 F-box proteins to direct selective ubiquitination (Guo and Ecker, 2003; Potuschak et al., 2003; Gagne et al., 2004). Ethylene appears to block this step, thus allowing EIN3 levels to rise. EIN3 and EIL1 are required for prolonged responses to ethylene, as evidenced by the observation that the *ein3-1 eil1-1* double loss-of-function mutant has no response to long ethylene treatments using end-point analysis of growth (Alonso et al., 2003). However, not all effects of ethylene are dependent upon EIN3 and EIL1. Detailed growth kinetics of etiolated Arabidopsis seedlings show that there are two phases to growth inhibition by ethylene (Binder et al., 2004a, 2004b). The first transient phase occurs even at very low levels of ethylene and is independent of EIN3 and EIL1, whereas the second, EIN3/EIL1-dependent phase is less sensitive to ethylene, slower in onset, and lasts as long as ethylene is applied.

Whereas a great deal of detail is now available about the effects of ethylene on growth, less is known about the kinetics and dose-response characteristics of other responses to ethylene in etiolated seedlings. In pea (*Pisum sativum*), stem lateral expansion and growth inhibition appear to occur with similar kinetics (Nee et al., 1978; Eisinger et al., 1983). We initiated this study to examine the kinetics of apical hook closure and hypocotyl thickening in Arabidopsis seedlings treated with ethylene. However, while conducting these studies, we made the observation that ethylene stimulates nutations in the hypocotyls of etiolated seedlings. Nutations are oscillatory nodding or bending movements caused by localized differential growth (Berg and Peacock, 1992) that were originally termed circumnutations by Darwin and Darwin (1880). Using high-resolution, time-lapse imaging of seedlings growing in darkness, we monitored the kinetics of both growth inhibition and nutations in hypocotyls caused by the application of ethylene to wild-type and mutant seedlings. Using this approach, we found that the effects of ethylene on nutations could be distinguished pharmacologically and genetically from its inhibitory effects on hypocotyl growth. The role of auxin transport was examined.

RESULTS

Ethylene Stimulates Nutations in Etiolated Arabidopsis Seedlings

During prolonged treatments with ethylene, we made the observation that ethylene stimulated nutational bending in the root tip (data not shown) and the hypocotyls of etiolated Arabidopsis (Columbia [Col-0]) seedlings growing on a vertically orientated agar plate (Fig. 1). We have observed these movements of hypocotyls in over 70 Col-0 wild-type seedlings as well as in the hypocotyls of Wassilewskija (WS) wild-type seedlings (data not shown). When observed from the side, these movements appeared as oscillatory bending movements in the plane of the agar surface with the zone of bending approximately 0.8 mm below the apex of the hook.

By measuring the angle of the bend in each frame of the time series (Fig. 1), we were able to plot the time course of hypocotyl bending to examine the amplitude, period, and delay of nutations. Figure 2A shows examples of the time course of hypocotyl bending for etiolated seedlings treated with 10 μL L^{-1} ethylene (more examples for this and other conditions and mutants used can be found in Supplemental Data S1). Unless otherwise specified, 5 μM L-α-(2-amino ethoxyvinyl)-Gly (AVG) was included in the agar to block biosynthesis of ethylene by the seedlings. The delay in nutation onset ranged from 2.75 to 10.75 h after addition of ethylene, with the average delay being 6.25 h. This delay did not appear to be dependent on concentrations of ethylene between 3 nL L^{-1} and 10 μL L^{-1}

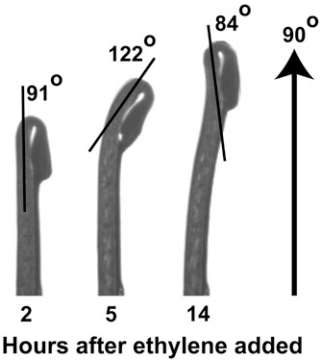

Figure 1. Ethylene stimulates nutations of etiolated Col-0 hypocotyls. Col-0 seedlings growing on vertically orientated plates were imaged with a close-focus, computer-driven camera with backlighting provided by an infrared LED equipped with a diffuser. For this and plots showing nutation angle over time, the angle of hypocotyls was determined by drawing a line from the region of bending in the hypocotyl to the apical hook. A protractor was used to manually measure the angle of this line. Vertical growth is defined as 90°, whereas angles >90° indicate the apical hook opening facing down and angles <90° indicate the hook opening facing up. The angle of the hypocotyl bend is shown above each hypocotyl. Images were taken at various times (shown below each image) after the addition of 10 μL L^{-1} ethylene.

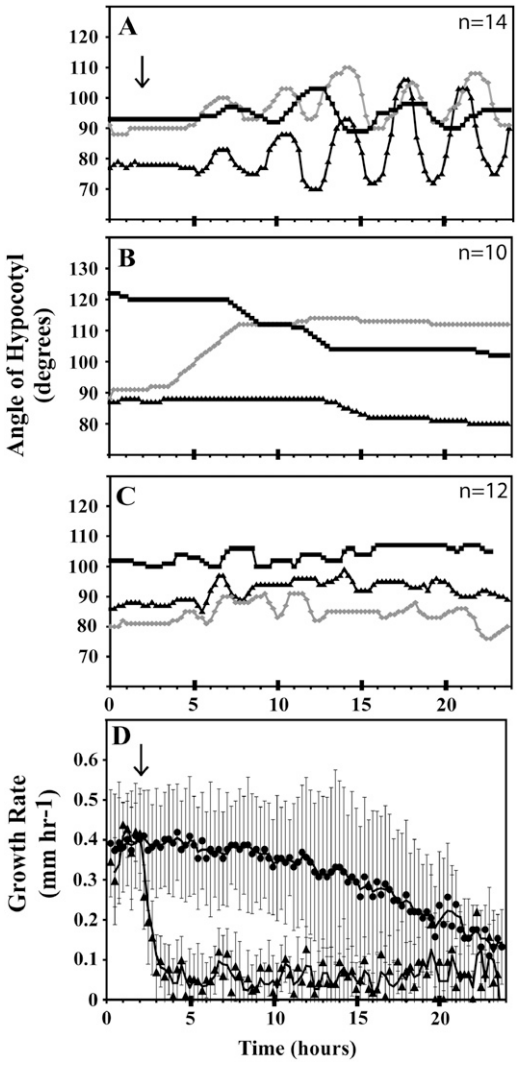

Figure 2. Ethylene stimulates nutations and inhibits growth of hypocotyls in etiolated Col-0 Arabidopsis seedlings. Col-0 wild-type hypocotyls were imaged from the side while growing along a vertically orientated agar plate. A to C, Hypocotyl angles were plotted as a function of time for three representative seedlings (n = the number of individual seedlings observed in each condition). Black and gray lines are used within each image to help distinguish the movements of individual seedlings. Except for C, all seedlings were grown in the presence of 5 μM AVG to block biosynthesis of ethylene by the seedlings. A, Seedlings were grown in air for 2 h prior to treatment with 10 μL L^{-1} ethylene (↓). B, Seedlings grew in air for 24 h without ethylene treatment. C, Seedlings were grown in air in the absence of AVG. D, Growth rate for seedlings grown in air (●) are compared to seedlings grown in air for 2 h prior to introducing 10 μL L^{-1} ethylene (▲) indicated by the arrow (↓). The growth rate curve for air treatment represents the average ± SD of 10 seedlings, whereas growth in ethylene represents the average ± SD of seven seedlings.

(data not shown). Some seedlings nutated in phase with each other even between different experiments or when the onset of nutation was delayed. The initial ethylene-stimulated nutation was almost always in the same direction relative to the opening of the apical hook, with the first bend causing the opening of the hook to face downward in over 90% of the seedlings observed. Nutations could occur quickly with rates of angle change approaching 1.3° min^{-1} for the higher amplitude oscillations.

To confirm that these movements were not a developmental change or artifact of the experimental system, we followed the growth and movement of etiolated seedlings in the absence of added ethylene. No nutations occurred in the presence of 5 μM AVG, which was included to inhibit ethylene biosynthesis (Fig. 2B). Sometimes, under this condition, very small (approximately 1°) and infrequent hypocotyl bending was observed. It was difficult to determine whether this was true nutational bending, bending due to movement over the agar surface, or due to some other factor. When AVG was omitted, small and frequent nutations were observed with an amplitude of 3.8° ± 2.4° (Fig. 2C). The period of these nutations was 2.6 ± 0.33 h, which is within the range previously reported for etiolated Arabidopsis hypocotyls (Orbović and Poff, 1997) and similar to the periods reported for Arabidopsis inflorescences (Hatakeda et al., 2003).

The growth inhibition response to ethylene was similar to that previously reported (Binder et al. 2004a, 2004b) and reached a steady-state growth rate of 0.05 ± 0.02 mm h^{-1} in the continued presence of 10 μL L^{-1} ethylene (Fig. 2D). Because we have not previously followed the growth rate of etiolated seedlings for longer than 12 h, we examined the growth rate of wild-type seedlings in air for 24 h. We found that the growth rate in air remained constant at approximately 0.41 mm h^{-1} for the first 12 h, but then slowly decreased to approximately 0.14 mm h^{-1} by 24 h after the start of observations (Fig. 2D).

Figure 3 shows the dose-response relationships for the period and amplitude of ethylene-stimulated nutations in Col-0 wild-type seedlings. The amplitude of hypocotyl nutations at various ethylene concentrations was determined by plotting nutation angle time courses and measuring the change in angle from each of the peaks to the midline of the sine wave. Nutation amplitude varied between 2.5° ± 1.3° and 12° ± 4.7° with a half-maximal response at approximately 40 nL L^{-1} ethylene. Nutation amplitude showed the largest changes between 10 and 100 nL L^{-1}. The response saturated at 100 nL L^{-1} with no further increase in nutation amplitude up to 10 μL L^{-1}. Thus, nutation amplitude was slightly more sensitive to ethylene than the long-term growth inhibition response previously reported for etiolated Arabidopsis hypocotyls (Chen and Bleecker, 1995; Hall et al., 1999; Gamble et al., 2002; Binder et al., 2004a). Nutation periodicity was calculated by measuring the time for one complete oscillation from peak to peak. Nutation periodicity showed a small variation with ethylene dosage ranging from 2.8 ± 0.3 h at 3 nL L^{-1} to 4.7 ± 1.0 h at 10 μL L^{-1} ethylene (Fig. 3).

Most experiments reported here were conducted on vertically orientated plates, which limits the nutational

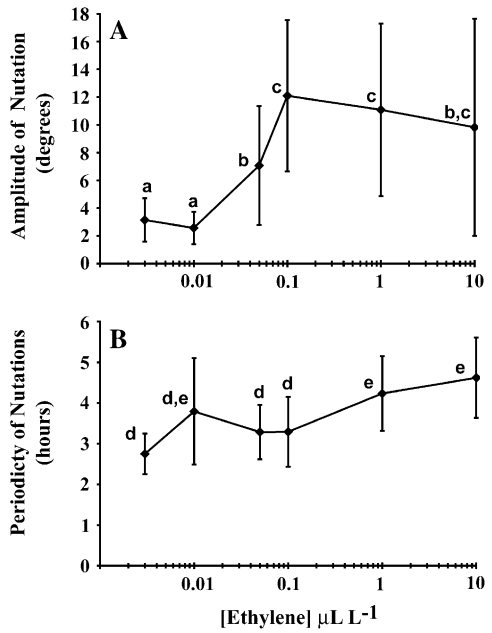

Figure 3. Ethylene dose-response relationship for the amplitude and period of hypocotyl nutations. A, The amplitude of hypocotyl nutations at various ethylene concentrations was determined by plotting nutation angle over time and measuring the change in angle from each peak to the midline of the sine wave. B, The period of nutations at various ethylene concentrations was determined by measuring the time for one complete oscillation in plots of nutation angle over time. Ethylene concentrations were determined by gas chromatography. Letters next to data points, when the same, indicate statistically insignificant differences. Differences were analyzed with *t* tests and considered statistically significant for $P < 0.05$. Tables with the statistics of the comparisons are available in Supplemental Data S1. All data represent the average ± SD for a total of at least seven seedlings in at least three separate experiments.

movements to the plane of the agar because the seedling is adhered to the agar surface. Nutational movement patterns can vary between species. Arabidopsis hypocotyls have been reported to nutate in a variety of patterns when not limited by physical constraints (Orbović and Poff, 1997; Schuster and Engelmann, 1997; Johnsson et al., 1999). To assess the nutation patterns of etiolated Arabidopsis hypocotyls, we imaged Col-0 wild-type seedlings from above while they grew on horizontally oriented plates in the presence of 1 μL L^{-1} ethylene (Fig. 4). Of the seven seedlings examined, six nutated in oval to circular patterns. Of these, two nutated clockwise and four nutated counterclockwise. One seedling initially nutated in a pendular side-to-side pattern, then switched to an elliptical, counterclockwise movement. This variation in movement pattern is in general agreement with previous results examining dark-grown (Orbović and Poff, 1997) and light-grown (Schuster and Engelmann, 1997) Arabidopsis seedling hypocotyls. These results indicate that ethylene at various concentrations can stimulate nutational bending in hypocotyls of etiolated Arabidopsis seedlings.

Mutations in the Ethylene-Signaling Pathway Alter Nutations

To confirm that these movements were due to the presence of ethylene, we studied various ethylene-sensing mutants. The constitutive ethylene response mutant, *ctr1-2*, nutated in air in the presence of AVG (Fig. 5A). The amplitude of these movements was 8.8° ± 3.0°, whereas the period was 4.2 ± 0.9 h. In contrast, treating the ethylene-insensitive mutant, *ein2-1*, with 10 μL L^{-1} ethylene failed to stimulate nutations (Fig. 5B). Similarly, ethylene-insensitive *etr1-1* mutants did not nutate (data not shown). The *ein2-1* mutants appeared partially agravitropic under the conditions used as evidenced by the prolonged deviation of growth from the gravity vector (90° in the plots) by many seedlings.

We have previously shown that plants lacking the EIN3 and EIL1 transcription factors have a transient growth inhibition response to ethylene (Binder et al., 2004a). To determine whether these transcription factors are also required for the nutations observed with long ethylene treatments, we treated *ein3-1 eil1-1* mutants with ethylene for 22 h (Fig. 5C). These mutants showed a transient growth response to ethylene (data not shown) as reported previously. In 67% of the *ein3-1 eil1-1* seedlings measured, ethylene failed to stimulate nutations. In double-mutant seedlings where nutations did occur, the amplitude of movement was small (2.7° ± 0.9°). Some of these mutant seedlings also appeared to be partially agravitropic under the conditions of these experiments.

Thus, components of the known ethylene-signaling pathway that lead to growth reduction in the hypocotyl are also involved in nutations stimulated by ethylene.

The ETR1 Receptor Is Required for Ethylene-Stimulated Nutations

We initiated experiments to examine the role of individual receptor isoforms in the nutation phenotype

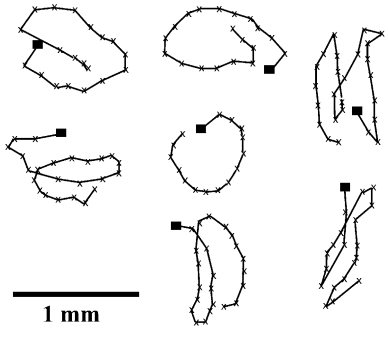

Figure 4. Hypocotyls nutate in a variety of patterns in the presence of ethylene. Individual Col-0 seedlings were imaged from above while growing in the presence of 1 μL L^{-1} ethylene on horizontally oriented plates. Images were captured every 15 min. The start position for each hypocotyl is marked with a black square. Positions at each 15-min interval thereafter are marked with an ×.

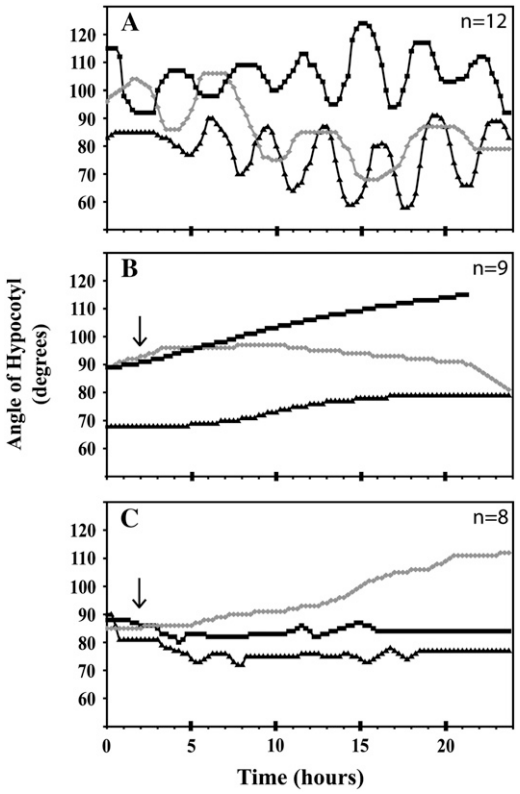

Figure 5. Ethylene-sensing mutants have altered nutation responses. Images of Arabidopsis hypocotyls growing along a vertically orientated agar plate were captured from the side every 15 min. Hypocotyl angles were plotted as a function of time for three representative seedlings of each mutant (n = total number of seedlings observed for each mutant). A, Mutant *ctr1-2* seedlings were grown in air. B, Mutant *ein2-1* seedlings. C, *ein3-1 eil1-1* double mutants were grown in air for 2 h before introducing 10 μL L^{-1} ethylene (↓). Black and gray lines are used within each image to help distinguish the movements of individual seedlings.

using loss-of-function mutants. Previously, it has been shown that single ethylene receptor loss-of-function mutants had little effect on growth or growth inhibition by ethylene, although the *etr1-7* loss-of-function mutant showed small, but measurable, growth changes in air and was slightly more sensitive to ethylene (Hua and Meyerowitz, 1998; Cancel and Larsen, 2002). We found that the *etr1-7* mutation was sufficient to eliminate nutations caused by application of ethylene (Fig. 6A). The growth rate in air and the response kinetics to ethylene in this mutant were similar to a previous study (Binder et al., 2004b). Nutations were rescued when the *etr1-7* mutant was transformed with a genomic *ETR1* transgene (Fig. 6B). Similarly, combinatorial receptor loss-of-function mutants that included loss of ETR1 function, such as the *etr1-7 ers1-2* double mutant and the *etr1-6 etr2-3 ein4-4* triple mutant, did not nutate (Fig. 6, C and E). These mutants were also rescued by transformation with a genomic *ETR1* transgene and displayed nutation amplitudes and periods indistinguishable from wild type (Fig. 6, D and F).

We examined loss-of-function mutants for the other four receptor isoforms to ascertain their roles in the nutation phenotype. The amplitude of nutations (Fig. 7A) and the extent of growth inhibition (Fig. 7B) in the presence of ethylene were similar to wild-type controls in both *ers1-2 ers2-3* double loss-of-function mutant seedlings and *etr2-3 ers2-3 ein4-4* triple loss-of-function mutants. Single loss-of-function mutations in these receptor isoforms had no measurable effect on nutations (data not shown). Because the *ers1-2* allele is not a complete loss-of-function mutant while the *ers1-3* allele appears to be a complete loss-of-function mutant (Xie et al., 2006), we also examined the *ers1-3* allele and still observed nutations, suggesting that only ETR1 is required for this phenotype (data not shown). The *etr2-3 ers2-3 ein4-4* triple mutant has previously been shown to have constitutive growth inhibition in air, although this growth inhibition was not as severe as the *ctr1-2* mutant (Hua and Meyerowitz, 1998; Zhao et al., 2002; O'Malley et al., 2005). We confirmed this constitutive growth inhibition under the conditions used in these experiments (Fig. 7B). This triple mutant also constitutively nutated in air in the presence of AVG with amplitudes somewhat smaller than those of the *ctr1-2* mutant (Fig. 7A). The *ers1-2 ers2-3* double mutant nutated constitutively in air even though no constitutive growth inhibition has been observed for this mutant (Fig. 7B; Hall and Bleecker, 2003). In air in the presence of AVG, both WS wild-type seedlings (control for the *ers1-2 ers2-3* double mutant) and Col-0 wild-type seedlings (control for the *etr2-3 ers2-3 ein4-4* triple mutant) grew at similar rates (Fig. 7B) and had small and infrequent hypocotyl bending. Whereas the amplitude of these bends in WS was larger than those of Col-0, they were infrequent in occurrence, making it uncertain whether or not they were true nutations. In the presence of 10 μL L^{-1} ethylene, the hypocotyls of WS seedlings nutated with a similar amplitude (Fig. 7A) and period (4.1 $\pm$ 0.7 h) as Col-0 seedlings.

These results are consistent with a model where all the ethylene receptor isoforms are involved in ethylene-stimulated nutations and the ETR1 receptor is required.

Auxin Transport Is Required for Ethylene-Stimulated Nutations

Because nutation has been linked to the plant hormone auxin (Britz and Galston, 1982a, 1982b, 1983), we tested the effects of the auxin transport inhibitor, naphthylphthalamic acid (NPA), on growth inhibition and nutations caused by the application of ethylene. NPA has previously been shown to eliminate nutations in the inflorescences of adult Arabidopsis (Hatakeda et al., 2003). When seedlings were transferred onto agar plates containing 5 μM NPA, the growth rate in air declined to approximately 66% of untreated seedlings. Addition of 10 μL L^{-1} ethylene caused growth inhibition with kinetics similar to that observed in the absence of NPA reaching a steady-state growth rate of

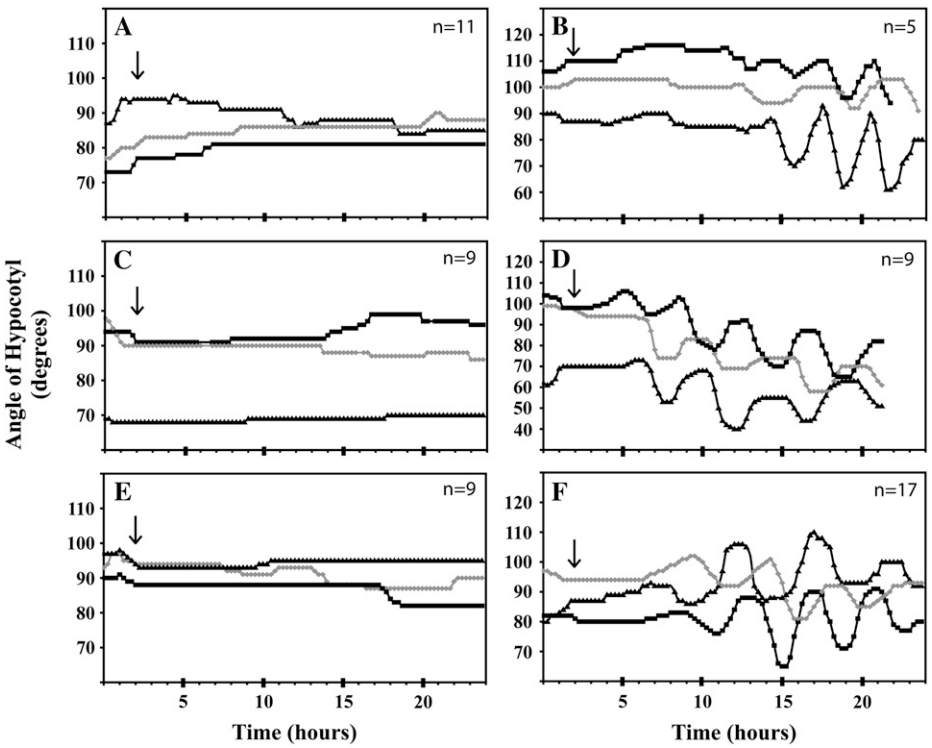

Figure 6. The ETR1 receptor is required for ethylene-stimulated nutations. Hypocotyl angles for three representative seedlings (n = total number of seedlings observed for each mutant and transformant) were plotted as a function of time for *etr1-7* (A), *etr1-7* transformed with a genomic *ETR1* transgene (B), *etr1-7 ers1-2* double mutant (C), *etr1-7 ers1-2* transformed with genomic *ETR1* (D), *etr1-6 etr2-3 ein4-4* triple mutant (E), and *etr1-6 etr2-3 ein4-4* transformed with genomic *ETR1* (F). Seedlings were maintained in air for 2 h before 10 μL L^{-1} ethylene were added (↓). Black and gray lines are used in each image to help distinguish the movements of individual seedlings.

0.07 ± 0.05 mm h^{-1} (Fig. 8A). NPA eliminated ethylene-stimulated nutations in 11 of 13 seedlings observed. The two seedlings that showed nutations only nutated for one cycle with very small amplitude (2.5°) movements (Fig. 8B). When the dosage of NPA was decreased to 1 μM, results were more variable, with some seedlings nutating with amplitudes and frequencies similar to untreated seedlings and others failing to nutate (data not shown).

DISCUSSION

Studies on the function of ethylene receptors have often focused on their role in the regulation of growth at the organ level. These studies indicate that the receptors have at least partially overlapping functions in growth regulation (Hua and Meyerowitz, 1998). Whereas the ethylene receptors are structurally related to bacterial two-component receptors that function by His autophosphorylation followed by phosphotransfer to a response regulator (West and Stock, 2001), this mechanism does not appear to be necessary for growth regulation mediated by the ethylene receptors (Wang et al., 2003; Binder et al., 2004b; Qu and Schaller, 2004). In this study, we find that ethylene leads to nutations in hypocotyls of etiolated Arabidopsis seedlings. This stimulation of nutations by ethylene let us study the function of the receptors in a new context. Unlike growth inhibition of the hypocotyl, these nutations require the ETR1 receptor, suggesting a special role for ETR1 in signaling leading to nutations.

Charles and Francis Darwin (1880) described nutations in plants over 125 years ago and these movements have been studied in many plant species, including Arabidopsis (Simmons et al., 1995; Marinelli et al., 1997; Orbović and Poff, 1997; Schuster and Engelmann, 1997; Mullen et al., 1998; Johnsson et al., 1999; Hatakeda et al., 2003; Piconese et al., 2003; Niinuma et al., 2005). Interestingly, Schuster and Engelmann (1997) observe nutations during growth bursts of the hypocotyl, whereas we observe nutations when growth of the hypocotyl is inhibited. The reason for this difference is unclear, but might be because we studied dark-grown seedlings, whereas Shuster and Engelmann studied light-grown seedlings. This stimulatory effect of ethylene on nutational movements in etiolated seedlings has not been previously published, although ethylene has been reported to induce tendril coiling in *Marah fabaceus* (Reinhold, 1967) and cucumber (*Cucumis sativus*; Bangerth, 1974) and to increase the amplitude and frequency of root waving in Arabidopsis roots, which might be linked to nutations (Buer et al., 2003). Additionally, ethylene has been proposed as a possible regulator of nutations, although no direct evidence for this was obtained at the time (Britz and Galston, 1982b). Early studies on the effects of ethylene applied to etiolated seedlings often mention horizontal nutation (Neljubov, 1901), but this refers to altered geotropism stimulated by ethylene (Knight and Crocker, 1913). Several lines of evidence support the idea that these nutations are initiated by ethylene. First, nutations in air are eliminated by the addition of AVG, which blocks ethylene biosynthesis. Second, nutational

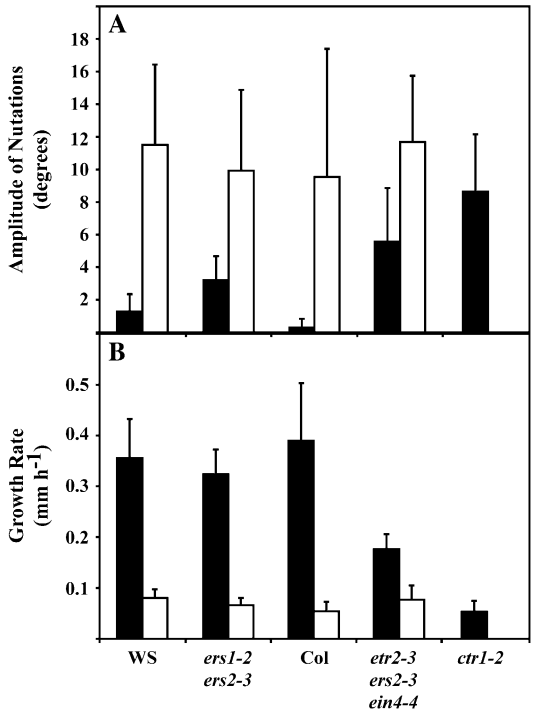

Figure 7. The ERS1, ERS2, ETR2, and EIN4 receptor isoforms are involved with, but not required for, ethylene-stimulated nutations. A, Amplitudes of hypocotyl nutations. B, Growth rates for seedlings maintained in air (black bars) or treated with 10 μL L^{-1} ethylene (white bars) are shown for *ers1-2 ers2-3* double mutants and *etr2-3 ers2-3 ein4-4* triple mutants. Col-0 wild-type seedlings were used as a control for the *etr2-3 ers2-3 ein4-4* triple mutants, whereas WS wild-type seedlings were used as a control for the *ers1-2 ers2-3* double mutants. Data from *ctr1-2* seedlings in air are shown for comparison. All data represent the average ± SD from at least four seedlings total from at least three separate experiments. Nutation amplitude was calculated as described in "Materials and Methods" and differences were analyzed with *t* tests. The nutation amplitude in air versus ethylene for each seed type was statistically different with $P < 0.003$. Nutation amplitude in air for each mutant versus its wild-type control was statistically different with $P < 0.001$. Growth rates in air were averaged from hypocotyl growth rates over the first 12 h from seedlings maintained in air, whereas growth rates in ethylene were averaged from hypocotyl growth rates between 3 and 12 h after ethylene was applied.

movements are constitutive in the *ctr1* mutant and the *etr2-3 ers2-3 ein4-4* triple mutant. Both mutants have constitutive growth inhibition in air. Finally, ethylene fails to stimulate nutations in the ethylene-insensitive *ein2* mutant.

There are differences that can be noted between the inhibitory effect of ethylene on hypocotyl growth and stimulation of nutations. The delay for long-term growth inhibition is approximately 1 h after application of ethylene and varies very little (Binder et al., 2004a, 2004b), whereas the average delay for nutations is over 6 h and varies by several hours. In addition, amplitude regulation of the nutation response is slightly more sensitive to ethylene than has been reported for the growth inhibition response in etiolated Arabidopsis hypocotyls (Chen and Bleecker, 1995; Hall et al., 1999; Gamble et al., 2002; Binder et al., 2004a). Whereas long-term growth inhibition by ethylene is eliminated in the *ein3 eil1* double mutant (Alonso et al., 2003, Binder et al., 2004a), ethylene sometimes stimulates small nutations in this mutant. This suggests that other components of the signaling pathway are still present downstream of EIN2 that are sufficient to support these movements. One candidate is another member of the EIN3 family of transcription factors, EIL2, which is likely to be involved in ethylene signaling. Because overexpression of EIL2 can complement the *ein3* loss-of-function mutant, including recovery of the growth response (Chao et al., 1997), it is also possible that EIL2 has a minor role in growth inhibition by ethylene that is below the detection limit of the methods used in our study.

We used receptor loss-of-function mutants to study the roles of the various receptor isoforms in ethylene signaling leading to nutations and growth inhibition. The results from these experiments further distinguish the inhibitor effect of ethylene on growth from its effect on nutations. In particular, the *etr1-7* loss-of-function mutant, but not other receptor mutants, fails to nutate in the presence of ethylene. Additionally, combinatorial receptor loss-of-function mutants that include loss of ETR1 function also do not nutate. The importance of ETR1 is further strengthened by the fact that transformation of these mutants with a genomic *ETR1* transgene rescues the nutation phenotype. In contrast, loss-of-function mutant combinations in the other receptor isoforms lead to constitutive growth

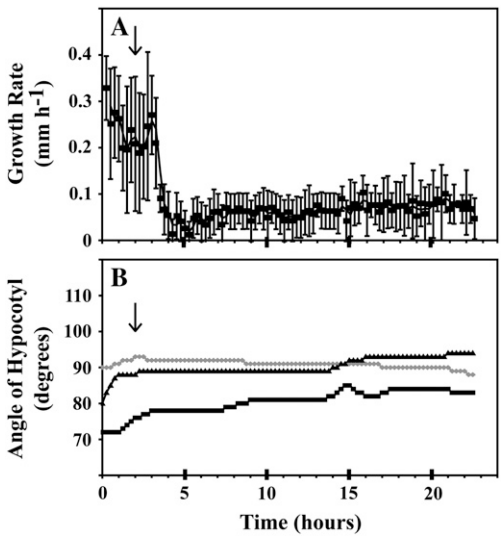

Figure 8. Auxin transport is required for ethylene-stimulated nutations but not growth inhibition. In both images, seedlings were transferred to plates containing 5 μM NPA to block auxin transport and grown in air for 3 h prior to the introduction of 10 μL L^{-1} ethylene (↓). Images of growing seedlings were captured every 15 min. A, The growth rate of seedlings is shown. Data represent the average ± SD for 12 seedlings. B, Hypocotyl angle for three seedlings are shown out of a total of 13 measured. Black and gray lines are used in B to help distinguish the movements of individual seedlings.

inhibition and nutations consistent with a model where all the receptors are involved in the signaling leading to ethylene-stimulated nutations. Previous work suggests that the five receptor isoforms have overlapping, but distinct, roles in signaling that lead to inhibition of growth (Chang et al., 1993; Hua et al., 1998; Hua and Meyerowitz, 1998; Sakai et al., 1998; Hall and Bleecker, 2003; Wang et al., 2003). The results in this study suggest a nonoverlapping function for ETR1 in the nutation phenotype.

Current models of ethylene signaling posit that receptors stimulate CTR1, which in turn acts as a negative regulator of the response pathway. In these models, ethylene inhibits receptor output, releasing the inhibition by CTR1. Hence, loss of ethylene receptors mimics the action of ethylene and causes constitutive ethylene responses in air. The observation that the *etr1-7* mutant fails to nutate is intriguing because this is opposite to what is predicted by these models. One way to reconcile this discrepancy is to invoke a model where ethylene induces differential expression of ETR1 in the zone of bending (see models in Supplemental Data S1). Because eliminating the ETR1 isoform results in lowered growth (Hua and Meyerowitz, 1998; Cancel and Larsen, 2002), it is possible that lower expression of ETR1 on one side of the hypocotyl relative to the other side would lead to differential growth rates resulting in bending. In this model, ethylene causes the expression of ETR1 to change in a localized region of the zone of bending. Over time, the localized region of altered ETR1 expression moves causing nutational oscillations. Whereas a previous study showed that ethylene did not cause alterations in overall *ETR1* expression in etiolated Arabidopsis seedlings (Binder et al., 2004b), it is possible that small, localized changes in transcript levels occurred below the detection limit of the method used.

An alternative model to explain the loss of nutations in the *etr1-7* mutants proposes that ETR1 has two functions, with one function regulating growth and the other supporting nutations (see Supplemental Data S1 for models). In this model, because the *etr1-7* mutation is not leading to constitutive nutations, it appears that ethylene is not acting to inhibit ETR1 to cause nutations as predicted from numerous other studies on ethylene signaling. Two alternative possibilities are that ETR1 is being stimulated by ethylene or that ETR1 is acting independently of ethylene to cause nutations. Whereas an ethylene-independent role in promoting cell elongation has been proposed for ETR1 (Hua and Meyerowitz, 1998), it seems unlikely that ETR1 is both inhibited (leading to most responses) and stimulated (leading to nutations) by ethylene over the same range of concentrations. However, *etr1-1* does not show the nutation phenotype. This mutant protein does not bind ethylene when expressed in yeast (*Saccharomyces cerevisiae*; Schaller et al., 1995; Rodriguez et al., 1999) and confers dominant ethylene insensitivity on plants (Bleecker et al., 1988; Chang et al., 1993). This suggests that ethylene binding to ETR1 may be required for signaling leading to nutations. Alternatively, the nutation phenotype may require both the presence of ETR1 acting in an ethylene-independent manner and growth inhibition caused by signaling through the receptors and CTR1. In this model, the *etr1-1* mutant does not nutate because growth inhibition does not occur. Whether both functions of ETR1 involve output to CTR1 is an open question. Whereas the existence of a CTR1-independent pathway has been proposed to explain the observation that *ctr1* loss-of-function mutants still respond to ethylene (Roman et al., 1995; Larsen and Chang, 2001), components in such a pathway have not been conclusively identified. If a second pathway is not involved, it means that CTR1 and EIN2 would also be bifunctional to support signaling from ETR1 that leads to both growth inhibition and nutations. Whereas evidence exists for a bifunctional EIN2 (Alonso et al., 1999; Binder et al., 2004a), no such evidence has been published for CTR1. The exact nature of this unique role for ETR1 in the nutation phenotype is unknown, but it is possible that there is a structural difference between ETR1 and the other receptor isoforms. One obvious structural difference between ETR1 and the other receptors is that it contains both a functional His kinase and a receiver domain. The γ-loop of the receiver domain is thought to be important in molecular interactions. This domain is located next to the conserved Asp-659 and in ETR1 has an atypical orientation compared to other receiver domains that have been structurally characterized (Müller-Dieckmann et al., 1999). This loop could function in coupling between the receiver domain of the ETR1 receptor and downstream signaling components. Thus, the output of ETR1 might be qualitatively different or this structure might lead to specific interactions lacking for the other receptor isoforms. There is precedence for believing that ETR1 has unique interactions with downstream components. Yeast two-hybrid experiments showed that ETR1, but not ERS1, interacts with the His-containing, phosphotransfer proteins ATHP1, 2, and 3 (Urao et al., 2000). Also, CTR1 interacts with ETR1, ERS1, and ETR2, but has the strongest interaction with ETR1 (Clark et al., 1998; Cancel and Larsen, 2002; Gao et al., 2003).

The downstream events by which ethylene causes nutations are not known. Auxin transport inhibitors, such as NPA, have been shown to block nutations in plants, including Arabidopsis (Hatakeda et al., 2003). Similarly, we find that NPA blocks nutations but has no measurable effect on growth inhibition caused by ethylene. Others have also separated overall growth from changes in growth that lead to nutations. For instance, NPA inhibited nutations but had a small effect on growth in stems of pea (Britz and Galston, 1983), and lithium chloride prevented nutations but did not alter growth in *Phaseolus vulgaris* shoots (Millet et al., 1984). This supports the assertion that, whereas nutations require growth, growth does not require nutations. Because ethylene can alter auxin distribution

and levels (Abeles, 1972), we hypothesize that, under the conditions used in these experiments, ethylene is stimulating nutations by differentially altering local auxin levels in the zone where nutational bending is observed. This would in turn cause a localized change in growth leading to a bend. It is an open question whether ethylene is leading to nutations outside the experimental environment used in our study. Bangerth (1974) found that both auxin and ethylene stimulate coiling of cucumber tendrils. In Bangerth's study, auxin was found to stimulate ethylene synthesis on one side of the tendril, which might be the cause for differential growth leading to coiling. Given the complexity of interactions between hormones in plants, it is also likely that other hormones could be involved with ethylene-stimulated nutations. At the cellular level, regulation of microtubule orientation has been put forth as a mechanism controlling nutations (Furutani et al., 2000). Ethylene affects microtubule orientation (Steen and Chadwick, 1981), suggesting another potential mechanism by which ethylene could regulate nutations. It should be noted, however, that many hormones affect microtubules (for review, see Shiboaka, 1994). Nutations have been modeled to involve interactions between internal and external factors, leading to nutational oscillations (Johnsson et al., 1999). Our data support the proposal of Britz and Galston (1982b) that ethylene might be one of the endogenous factors involved with regulation of nutations.

The role for nutations in etiolated seedlings is not known, although as early as the 1880s it was postulated that these movements could help with penetration through the soil (Darwin and Darwin, 1880). In rice (*Oryza sativa*), nutation of roots has been reported to increase soil penetration (Inoue et al., 1999). Whether this is true for other species or for the hypocotyl is unknown. Production of ethylene increases when plants undergo mechanical stimulation (Goeschl et al., 1966; Biro and Jaffe, 1984; Takahashi and Jaffe, 1984; Whalen, 1988; Hussain et al., 1999; Roberts et al., 2002), suggesting that the triple response coupled with nutational movements might aid the seedling to better penetrate and navigate through the soil.

MATERIALS AND METHODS

AVG was kindly supplied by Rohm Haas, Inc. and NPA came from Sigma (St. Louis). The *etr1-7*, *etr2-3*, *ers2-3*, and *ein4-4* mutants were obtained from Elliot Meyerowitz (Hua and Meyerowitz, 1998). The *ctr1-2* mutant was from Joseph Kieber (Kieber et al., 1993) and the *ein2-1* mutant from Joseph Ecker (Alonso et al., 1999). The *ers1-2* mutant was isolated in this lab and described previously (Wang et al., 2003; Hall and Bleecker, 2003). Mutants were in the Col-0 background, except for *ers1-2* and *ers2-3*, which were in the WS background. The *etr1-7 ers1-2* and *etr1-6 ers2-3 ein4-4* mutants transformed with a genomic *ETR1* transgene were generated previously and are described in Wang et al. (2003) and Binder et al. (2004b). The *etr1-7* mutant line transformed with genomic the *ETR1* transgene was kindly supplied by Eric Schaller and is described in Gamble et al. (2002).

Seedling Preparation

Arabidopsis (*Arabidopsis thaliana*) seeds were surface sterilized by treatment with 70% alcohol for 30 s, placed on sterile filter paper to dry, and then placed on agar plates containing one-half-strength Murashige and Skoog basal salt mixture, pH 5.7 (Murashige and Skoog, 1962), 0.8% agar, and B5 vitamins consisting of inositol (100 mg mL^{-1}), nicotinic acid (1 mg mL^{-1}), pyridoxin HCl (1 mg mL^{-1}), and thiamine HCl (10 mg mL^{-1}) with no added sugar. Unless otherwise specified, 5 μM AVG were added to inhibit ethylene production by the seedlings. Seeds were treated for 2 to 4 d at 4°C, light treated for 4 to 8 h under continuous fluorescent lights, and then grown in darkness at 22°C to be used for growth kinetics measurements.

Time-Lapse Imaging

Seedlings were allowed to grow on vertically orientated plates in darkness to a height of 2 to 4 mm (40–46 h) before the beginning of measurements, as modified from Binder et al. (2004a, 2004b). Briefly, following 2 h of treatment with air to establish a basal growth rate and movement pattern, ethylene was introduced to the chamber at a flow rate of 10 mL min^{-1}. The experimental chamber maintained the agar plate in vertical orientation with no measurable tilt relative to the gravity vector. We have previously determined that the treatment chamber equilibrates to a steady-state ethylene concentration within 5 min (Binder et al., 2004b). Seedlings were exposed to ethylene for 22 h. Unless otherwise specified, an ethylene concentration of 10 μL L^{-1} was used. Ethylene concentrations were determined by gas chromatography as previously described (Binder et al., 2004a, 2004b). Control experiments were conducted where ethylene was not introduced into the experimental chamber. In experiments involving NPA, seedlings were transferred to plates containing 5 μM NPA and allowed to grow in air for 3 h prior to exposure to ethylene for 21 h. All manipulations were done under dim green illumination.

Images of hypocotyls of etiolated seedlings were captured using infrared radiation, an electronic camera from either Electrim or Luminera, and custom software as modified from previous studies (Parks and Spalding, 1999; Folta and Spalding, 2001; Binder et al., 2004a, 2004b). Electronic images were captured every 15 min for 24 h with light for imaging produced by an infrared light-emitting diode positioned behind the seedlings and equipped with a reflective diffuser. For these experiments, image resolution was quantified in every experiment and ranged between 33 and 90 pixels per millimeter.

Analysis of Growth Rate and Nutation Angles in Hypocotyls

To determine the growth rate of the hypocotyls, one of two methods was used. In one, the height in pixels of each seedling in each frame was analyzed using custom software written by Edgar Spalding in LabVIEW 5.0 (National Instruments) as previously described (Parks and Spalding, 1999; Folta and Spalding, 2001). During nutational movements, this method was inaccurate due to the movement of the hypocotyls. In this case, the length of the hypocotyl in each frame was measured manually using the computer program Image J (http://rsb.info.nih.gov/ij). From both methods, we calculated growth rates for each 15-min increment using Microsoft Excel.

Nutation angles of hypocotyls were measured manually. For each frame in a time series, a line was drawn from the hypocotyl bend to the apical hook (Fig. 1) and a protractor was used to measure the angle of this line. These data were plotted as a function of time. In these plots, 90° indicates growth directly against the gravity vector, whereas angles >90° indicate the opening of the apical hook is aimed down and <90° that the opening is aimed up. Seedlings sometimes grew at vectors other than directly against gravity; this is reflected by the prolonged angle of growth deviating from 90°. Representative seedlings were chosen for figures showing nutation angle over time. The total number (n) of seedlings observed in each condition is shown in each nutation time course. See Supplemental Data S1 for additional nutation time-course plots. Experiments under all conditions were repeated in at least four separate experiments.

Because nutations represent a sinusoidal oscillation of bending over time, the nutation amplitude was determined by measuring the change in angle from the peak of each oscillation to the midline of the sine wave. The periodicity of nutations was determined by measuring the time for one complete oscillation for each cycle. We measured this by determining the time between each peak of movement. These measurements were analyzed using t tests with $P < 0.05$ considered statistically significant.

Measurement of Nutation Shape

In two experiments, the shape and direction of nutations were determined by growing plants on horizontal plates and imaging growing seedlings from

above. Images were captured every 15 min and the pattern of movement determined by marking the position of the hypocotyl in each frame on transparency film. These traces were then scanned and transferred into Adobe Illustrator.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Data S1. Additional nutation time courses, statistics, and models of signaling.

ACKNOWLEDGMENTS

We thank Edgar Spalding for helpful advice concerning this work, and Caren Chang, Mark Estelle, and Sara Patterson for critiques of early versions of this manuscript. We also thank Eric Schaller for the *ers1-3* mutant seeds and the *etr1-7* mutant line transformed with genomic *ETR1* transgene.

Received August 2, 2006; accepted October 17, 2006; published October 27, 2006.

LITERATURE CITED

Abeles FB (1972) Biosynthesis and mechanism of action of ethylene. Annu Rev Plant Physiol **23:** 259–292

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in Plant Biology, Ed 2. Academic Press, San Diego

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. Science **284:** 2148–2152

Alonso JM, Stepanova AN, Solano R, Wisman E, Ferrari S, Ausubel FM, Ecker JR (2003) Five components of the ethylene-response pathway identified in a screen of weak ethylene-insensitive mutants in *Arabidopsis*. Proc Natl Acad Sci USA **100:** 2992–2997

Bangerth F (1974) Interaktionen von Auxin und Äthylen bei der thigmotropen Bewegung der Ranken von *Cucumis sativus*. Planta **117:** 329–338

Berg AR, Peacock K (1992) Growth patterns in nutating and nonnutating sunflower (*Helianthus annuus*) hypocotyls. Am J Bot **79:** 77–85

Binder BM, Mortimore LA, Stepanova AN, Ecker JR, Bleecker AB (2004a) Short term growth responses to ethylene in Arabidopsis seedlings are EIN3 /EIL1 independent. Plant Physiol **136:** 2921–2927

Binder BM, O'Malley RC, Wang W, Moore JM, Parks BM, Spalding EP, Bleecker AB (2004b) Arabidopsis seedling growth response and recovery to ethylene: a kinetic analysis. Plant Physiol **136:** 2913–2920

Biro RL, Jaffe MJ (1984) Thigmomorphogenesis: ethylene evolution and its role in the changes observed in mechanically perturbed bean plants. Physiol Plant **62:** 289–296

Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science **241:** 1086–1089

Britz SJ, Galston AW (1982a) Physiology of movements in stems of seedling *Pisum sativum* L. cv. Alaska. I. Experimental separation of nutation from gravitropism. Plant Physiol **70:** 264–271

Britz SJ, Galston AW (1982b) Physiology of movements in stems of seedling *Pisum sativum* L. cv. Alaska. II. The role of the apical hook and of auxin in nutation. Plant Physiol **70:** 1401–1404

Britz SJ, Galston AW (1983) Physiology of movements in stems of seedling *Pisum sativum* L. cv. Alaska. III. Phototropism in relation to gravitropism, nutation, and growth. Plant Physiol **71:** 313–318

Buer CS, Wasteneys GO, Masle J (2003) Ethylene modulates root-wave responses in Arabidopsis. Plant Physiol **132:** 1085–1096

Cancel JD, Larsen PB (2002) Loss-of-function mutations in the ethylene receptor *ETR1* cause enhanced sensitivity and exaggerated response to ethylene in Arabidopsis. Plant Physiol **129:** 1557–1567

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) *Arabidopsis* ethylene-response gene ETR1: similarity of product to two-component regulators. Science **262:** 539–543

Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR (1997) Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell **89:** 1133–1144

Chen QC, Bleecker AB (1995) Analysis of ethylene signal-transduction kinetics associated with seedling-growth response and chitinase induction in wild-type and mutant Arabidopsis. Plant Physiol **108:** 597–607

Chen Y-F, Etheridge N, Schaller GE (2005) Ethylene signal transduction. Ann Bot (Lond) **95:** 901–915

Clark KL, Larsen PB, Wang X, Chang C (1998) Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS1 ethylene receptors. Proc Natl Acad Sci USA **95:** 5401–5406

Darwin C, Darwin F (1880) The Power of Movement in Plants. John Murray, London

Eisinger WR, Croner L, Taiz L (1983) Ethylene-induced lateral expansion in etiolated pea epicotyls: analysis of kinetics. Plant Physiol **73:** 407–412

Folta KM, Spalding EP (2001) Unexpected roles for cryptochrome 2 and phototropin revealed by high-resolution analysis of blue light-mediated hypocotyl growth inhibition. Plant J **26:** 471–478

Furutani I, Watanabe Y, Prieto R, Masukawa M, Suzuki K, Naoi K, Thitamadee S, Shikanai T, Hashimoto T (2000) The *SPIRAL* genes are required for directional control of cell elongation in *Arabidopsis thaliana*. Development **127:** 4443–4453

Gagne JM, Smalle J, Gingerich DJ, Walker JM, Yoo S-D, Yanagisawa S, Vierstra RD (2004) *Arabidopsis* EIN3-binding F-box 1 and 2 form ubiquitin-protein ligases that repress ethylene action and promote growth by directing EIN3 degradation. Proc Natl Acad Sci USA **101:** 6803–6808

Gamble RL, Coonfield ML, Schaller GE (1998) Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. Proc Natl Acad Sci USA **95:** 7825–7829

Gamble RL, Qu X, Schaller GE (2002) Mutational analysis of the ethylene receptor ETR1. Role of the histidine kinase domain in dominant ethylene insensitivity. Plant Physiol **128:** 1428–1438

Gao Z, Chen Y-F, Randlett MD, Zhao X-C, Findell JL, Kieber JJ, Schaller GE (2003) Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of *Arabidopsis* through participation in ethylene receptor signaling complexes. J Biol Chem **278:** 34725–34732

Goeschl JD, Rappaport L, Pratt HK (1966) Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. Plant Physiol **41:** 877–884

Guo H, Ecker JR (2003) Plant responses to ethylene gas are mediated by $SCF^{EBF1/EFB2}$-dependent proteolysis of EIN3 transcription factor. Cell **115:** 667–677

Guo H, Ecker JR (2004) The ethylene signaling pathway: new insights. Curr Opin Plant Biol **7:** 40–49

Hall AE, Bleecker AB (2003) Analysis of combinatorial loss-of-function mutants in the Arabidopsis ethylene receptors reveals that the *ers1 etr1* double mutant has severe developmental defects that are EIN2 dependent. Plant Cell **15:** 2032–2041

Hall AE, Chen QG, Findell JL, Schaller GE, Bleecker AB (1999) The relationship between ethylene binding and dominant insensitivity conferred by mutant forms of the ETR1 ethylene receptor. Plant Physiol **121:** 291–299

Hatakeda Y, Kamada M, Goto N, Fukaki H, Tasaka M, Suge H, Takahashi H (2003) Gravitropic response plays an important role in the nutational movements of the shoots of *Pharbitis nil* and *Arabidopsis thaliana*. Physiol Plant **118:** 464–473

Hua J, Chang C, Sun Q, Meyerowitz EM (1995) Ethylene insensitivity conferred by *Arabidopsis* ERS gene. Science **269:** 1712–1714

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell **94:** 261–271

Hua J, Sakai H, Nourizadeh S, Chen QG, Bleecker AB, Ecker JR, Meyerowitz EM (1998) EIN4 and ERS2 are members of the putative ethylene receptor gene family in Arabidopsis. Plant Cell **10:** 1321–1332

Huang Y, Li H, Hutchison CE, Laskey J, Kieber JJ (2003) Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in *Arabidopsis*. Plant J **33:** 221–233

Hussain A, Black CR, Taylor IB, Roberts JA (1999) Soil compaction. A role for ethylene in regulating leaf expansion and shoot growth in tomato. Plant Physiol **121:** 1227–1237

Inoue N, Arase T, Hagiwara M, Manao T, Hyashi T, Ikeda R (1999) Ecological significance of root tip rotation for seedling establishment of *Oryza sativa* L. Ecol Res **14:** 31–38

Johnsson A, Jansen C, Engelmann W, Schuster J (1999) Circumnutations without gravity: a two-oscillator model. J Gravit Physiol **6:** 9–12

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. Cell **72:** 427–441

Knight LI, Crocker W (1913) Toxicity of smoke. Bot Gaz **55:** 337–371

Larsen PB, Chang C (2001) The Arabidopsis *eer1* mutant has enhanced ethylene responses in the hypocotyl and stem. Plant Physiol **125:** 1061–1073

Marinelli B, Gomarasca S, Soave C (1997) A pleiotropic *Arabidopsis thaliana* mutant with inverted root chirality. Planta **202:** 196–205

Millet B, Melin D, Bonnet B, Ibrahim CA, Mercier J (1984) Rhythmic circumnutation movement of the shoots in *Phaseolus vulgaris* L. Chronobiol Int **1:** 11–19

Moussatche P, Klee H (2004) Autophosphorylation activity of the *Arabidopsis* ethylene receptor multigene family. J Biol Chem **279:** 48734–48741

Mullen JL, Turk E, Johnson K, Wolverton C, Ishikawa H, Simmons C, Soll D, Evans ML (1998) Root-growth behavior of the Arabidopsis mutant *rgr1*: roles of gravitropism and circumnutation in the waving/coiling phenomenon. Plant Physiol **118:** 1139–1145

Müller-Dieckmann H-J, Grantz AA, Kim S-H (1999) The structure of the signal receiver domain of the *Arabidopsis thaliana* ethylene receptor ETR1. Structure **7:** 1547–1556

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant **15:** 473–497

Nee M, Chiu L, Eisinger W (1978) Induction of swelling in pea internode tissue by ethylene. Plant Physiol **62:** 902–906

Neljubov D (1901) Uber die horizontale Nutation der Stengel von *Pisum sativum* und einiger Anderer. Pflanzen Beih Bot Zentralb **10:** 128–139

Niinuma K, Someya N, Kimura M, Yamaguchi I, Hamamoro H (2005) Circadian rhythm of circumnutation in inflorescence stems of *Arabidopsis*. Plant Cell Physiol **46:** 1423–1427

O'Malley RC, Rodriguez FI, Esch JJ, Binder BM, O'Donnell P, Klee HJ, Bleecker AB (2005) Ethylene-binding activity, gene-expression levels, and receptor-system output for ethylene-receptor family members from *Arabidopsis* and tomato. Plant J **41:** 651–659

Orbović V, Poff KL (1997) Interaction of light and gravitropism with nutation of hypocotyls of *Arabidopsis thaliana* seedlings. Plant Growth Regul **23:** 141–146

Parks BM, Spalding EP (1999) Sequential and coordinated action of phytochromes A and B during Arabidopsis stem growth revealed by kinetic analysis. Proc Natl Acad Sci USA **96:** 14142–14146

Piconese S, Tronelli G, Pippia P, Migliaccio F (2003) Chiral and non-chiral nutations in Arabidopsis roots grown on the random positioning machine. J Exp Bot **54:** 1909–1918

Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, Genschik P (2003) EIN3-dependent regulation of plant ethylene hormone signaling by two *Arabidopsis* F box proteins: EBF1 and EBF2. Cell **115:** 679–689

Qu X, Schaller GE (2004) Requirement of the histidine kinase domain for signal transduction by the ethylene receptor ETR1. Plant Physiol **136:** 2961–2970

Reinhold L (1967) Induction of coiling in tendrils by auxin and carbon dioxide. Science **158:** 791–793

Roberts JA, Hussain Y, Taylor IB, Black CR (2002) Use of mutants to study long-distance signalling. J Exp Bot **53:** 45–50

Rodriguez F, Esch J, Hall A, Binder B, Schaller GE, Bleecker AB (1999) A copper cofactor for the ETR1 receptor from Arabidopsis. Science **283:** 996–998

Roman G, Lubarsky B, Kieber JJ, Rothenberg M, Ecker JR (1995) Genetic analysis of ethylene signal transduction in Arabidopsis thaliana: five novel mutant loci integrated into a stress response pathway. Genetics **139:** 1393–409

Sakai H, Hua J, Chen QG, Chang C, Medrano LJ, Bleecker AB, Meyerowitz EM (1998) *ETR2* is an *ETR1*-like gene involved in ethylene signaling in *Arabidopsis*. Proc Natl Acad Sci USA **95:** 5812–5817

Schaller GE, Ladd AN, Lanahan MB, Spanbauer JM, Bleecker AB (1995) The ethylene response mediator ETR1 from *Arabidopsis* forms a disulfide-linked dimer. J Biol Chem **270:** 12526–12530

Schuster J, Engelmann W (1997) Circumnutations of *Arabidopsis thaliana* seedlings. Biol Rhythm Res **28:** 422–440

Shiboaka H (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. Annu Rev Plant Physiol Plant Mol Biol **45:** 143–150

Simmons C, Soll D, Migliaccio F (1995) Circumnutations and gravitropism cause root waving in *Arabidopsis thaliana*. J Exp Bot **46:** 143–150

Solano R, Stepanova A, Chao Q, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. Genes Dev **12:** 3703–3714

Steen DA, Chadwick AV (1981) Ethylene effects in pea stem tissue: evidence of microtubule mediation. Plant Physiol **67:** 460–466

Takahashi H, Jaffe MJ (1984) Thigmomorphogenesis: the relationship of mechanical perturbation to elicitor-like activity and ethylene production. Physiol Plant **61:** 405–411

Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K (2000) Possible His to Asp phosphorelay signaling in an *Arabidopsis* two-component system. FEBS Lett **478:** 227–232

Wang W, Hall AE, O'Malley RC, Bleecker AB (2003) Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from *Arabidopsis* is not required for signal transmission. Proc Natl Acad Sci USA **100:** 352–357

West AH, Stock AM (2001) Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci **26:** 369–376

Whalen MC (1988) The effect of mechanical impedance on ethylene production by maize roots. Can J Bot **66:** 2139–2142

Xie F, Liu Q, Wen C-K (2006) Receptor signal output mediated by the ETR1 N-terminus is primarily subfamily I receptor dependent. Plant Physiol **142:** 492–508

Yanagisawa S, Yoo S-D, Sheen J (2003) Differential regulation of EIN3 stability by glucose and ethylene signaling in plants. Nature **425:** 521–525

Zhao X-C, Qu X, Mathews DE, Schaller GE (2002) Effect of ethylene pathway mutations upon expression of the ethylene receptor ETR1 from Arabidopsis. Plant Physiol **130:** 1983–1991