

Ethylene: a gaseous signal in plants and bacteria

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Ethylene was the first gaseous growth regulator discovered due to its pronounced effects on plant growth and development. Besides plants, many bacteria also have ethylene-binding proteins, indicating that the ability to bind and respond to ethylene is an evolutionarily ancient sensory mechanism. The recent characterization of an ethylene receptor from cyanobacteria and the finding that it plays a role in phototaxis confirms a prokaryotic role for the ethylene receptors and is consistent with the hypothesis that plants acquired ethylene receptors from the endosymbiont that gave rise to the chloroplast. The signalling pathway acting downstream of the plant ethylene receptors is considerably diverged from that found in bacteria, pointing to adaptations that can occur in transitioning from a prokaryotic to a eukaryotic cellular environment. Interestingly, although pathways for ethylene biosynthesis and signalling are conserved in plant lineages extending back to the green algae, there are examples of plants where these pathways have been lost, with ethylene no longer playing a regulatory role.

A brief history of ethylene as a plant hormone

The saying “one bad apple spoils the whole bunch” has a real biological basis. The gaseous hydrocarbon ethylene (C_2H_4) is most commonly known as the fruit-ripening hormone, and a rotting apple will release ethylene and thereby stimulate the ripening and rotting of nearby fruit. Although unaware of ethylene itself, farmers in ancient Egypt and the Middle East exploited its effects through their gashing of unripe sycamore figs, a practice that causes a burst of ethylene which then drives ripening. The modern fruit industry uses ethylene to ripen fruit in transit to stores so that it is shelf-ready when arriving at the market. Ethylene inhibitors, such as the chemical 1-methylcyclopropene (1-MCP) sold under various brand names, are used to preserve fruit. The fact that ethylene affects plant growth and inhibitors exist implies that plants can sense ethylene. Indeed, plants synthesize ethylene and sense it through specific receptors. Physiologically, ethylene has roles in cell elongation, organ abscission, senescence, pathogen resistance, abiotic stress, cell division, metabolism, flowering and fruit development, with many of these roles being of significant agronomic importance¹. Because of its gaseous nature, ethylene can diffuse rapidly throughout plant cells, cross cell membranes and escape the plant as an environmental signal.

The history of plant research on ethylene extends back for over a century to the Industrial Revolution, when observations suggested that illuminating gas was altering plant growth. Illuminating gas, a coal by-product piped throughout cities for street lamps in the 19th and

early 20th Centuries, would leak up to 10% of the gas. Plants near illuminating gas pipes were damaged or died due to inhibited root growth, accelerated senescence, leaf and petal abscission, and other alterations to normal growth. In 1901, Dimitry Neljubow demonstrated that the active component in illuminating gas was ethylene. In the 1930s, Richard Gane showed that plants synthesized their own ethylene by isolating detectable quantities from 27.2 kg (60 lb) of apples, thereby establishing ethylene as an endogenous plant hormone, the first gaseous growth regulator identified in any organism. The biochemical pathway for ethylene biosynthesis was elucidated during the 1960s and 1970s. It was not until the 1990s that the key elements in the ethylene signalling pathway were determined, this major advance taking place as a result of the elegant genetics made possible with the model plant *Arabidopsis* (Figure 1). For those interested, Bakshi et al.² provide an excellent historical overview of ethylene research.

Ancient origin of ethylene receptors

Observations of flowering plants led to the discovery of ethylene as a hormone, but the ability to sense ethylene is evolutionarily ancient, preceding the existence of plants. Sequencing of bacterial genomes has revealed genes that encode proteins with ethylene-binding domains similar to those of plant ethylene receptors. This is perhaps not too surprising in retrospect, because the signal output domains of plant ethylene receptors are related to two-component signalling elements of bacteria. At their simplest, two-component signalling systems involve a membrane-bound receptor that is phosphorylated on a

histidine residue in response to ligand binding (Figure 2). The phosphate is then transferred to an aspartic acid residue in a soluble response regulator, which then elicits downstream responses such as changes in gene expression. Eukaryotic two-component signalling systems are found in fungi, algae and plants³. The fact that ethylene-receptor-like proteins, with similar input and output domains, are found in bacteria and plants points to a remarkable conservation of structure across more than a billion years of evolution.

Recent studies with cyanobacteria demonstrate not only that bacteria contain sequences that look like ethylene receptors, but also that these are capable of binding ethylene and eliciting physiological responses, hallmarks for receptor function⁴. Cyanobacteria are blue-green bacteria capable of photosynthesis and responsible for oxygenating Earth's atmosphere two billion years ago. The receptor in question is found in the cyanobacterium *Synechocystis* and is named ETHYLENE RESPONSE1, or SynETR1 for short. SynETR1 contains two input domains: an ethylene-binding domain and a light-sensing domain (Figure 2). Ethylene and light appear to have opposing effects on signal output of the receptor, with UV-violet light inducing movement away from the light source (negative phototaxis) and ethylene inducing movement towards the light source (positive phototaxis). Ethylene also operates through SynETR1 to promote the formation of bacterial pili involved in bacterial movement. Signalling itself operates through a canonical two-component signalling system such that SynETR1 phosphorylates a response regulator, which in turn controls gene expression. Interestingly, although ethylene-producing bacteria exist, the *Synechocystis* strain with SynETR1 does not synthesize ethylene. This suggests that SynETR1 may be involved in detecting environmental ethylene or perhaps other hydrocarbons, not specifically ethylene. Ethylene is produced from organics exposed to sunlight, so the ability of SynETR1 to sense ethylene and light is likely to play a role in the cyanobacterium finding optimal photosynthesis conditions.

The identification of SynETR1 in cyanobacteria as a genuine ethylene receptor has significant implications for how plants acquired their repertoire of ethylene receptors. A likely hypothesis is that ethylene receptors were co-opted for signalling by higher plants during the endosymbiotic event by which an ancestral SynETR1-like containing cyanobacterium became a chloroplast. As described below, this probably occurred in the green algae that are land plant ancestors. As with many ancestral chloroplast genes, the gene encoding the ethylene receptor of the endosymbiont eventually moved to the nuclear genome of the host cell over evolutionary time. Alternatively, plant ethylene receptors may have their origin via a horizontal gene transfer to algae from a bacterium with an ethylene-receptor-like gene.

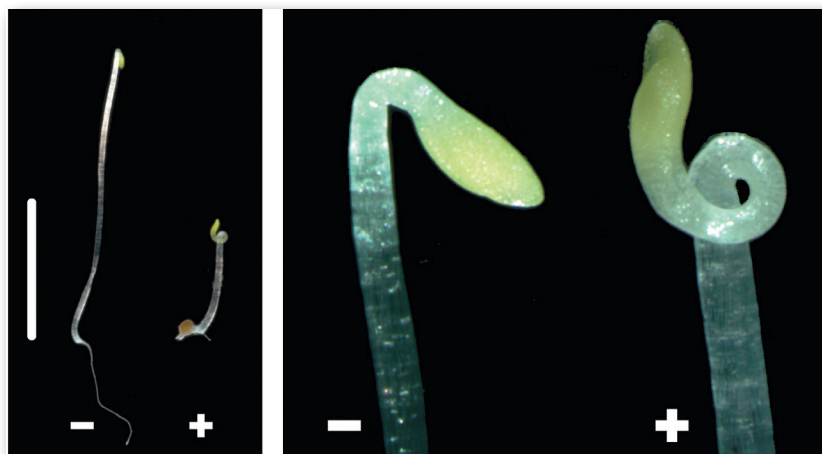


Figure 1. The morphological response to ethylene of dark-grown *Arabidopsis* seedlings. In response to ethylene, dark-grown *Arabidopsis* seedlings exhibit a shortening and thickening of the hypocotyl stem, an inhibition of root growth and the formation of an exaggerated apical hook. Seedlings are shown grown in the absence (–) or presence (+) of ethylene (scale bar, 5 mm). A close-up of the apical hook is shown on the right.

Pathways for ethylene biosynthesis and signalling exist in green algae

The functional conservation between the ethylene receptors of cyanobacteria and plants might suggest that plants would also rely on a two-component system to relay the ethylene signal. This, however, is not the case. Although evidence exists that signal output from the receptors can make use of a two-component-like signalling system, this is not the major signalling system operating downstream of the receptors⁵. In fact, the evolutionary trajectory of plant two-component elements frequently seems to move away from the original histidine/aspartic acid phosphorylation activities of two-component systems, with these elements then incorporated into the serine/threonine-phosphorylation framework prevalent in eukaryotes³. This is exemplified by the chimaeric nature of the plant ethylene signal transduction pathway (Figure 3). First, although some of the plant ethylene receptors have histidine kinase activity, others have diverged so much that, although they preserve a functional ethylene-binding domain, they lack histidine kinase activity; some in fact appear to have acquired serine/threonine kinase activity. In addition, the primary function of the histidine-kinase-like output domain of the receptors is now as a docking site for a serine/threonine kinase called CTR1, which belongs to the family of Raf-like kinases unique to eukaryotes. Other elements in the plant ethylene signalling pathway include the transmembrane protein EIN2, which also belongs to a eukaryote-specific protein family, and the plant-specific EIN3 family of transcription factors. The ethylene receptors, CTR1 and

EIN2 are predominantly localized to the membrane of the endoplasmic reticulum. This might be considered an unusual location for hormone perception, as the plasma membrane is the most logical location for hormone receptors, but internal localization is quite compatible with the cross-membrane diffusion of ethylene.

Given the differences between the 'modern' ethylene signalling pathway of plants and the 'ancient' two-component ethylene signalling pathway of bacteria, an important question to resolve is when plants acquired their suite of ethylene biosynthesis and signalling genes. The answer to this question has become increasingly tractable through analysis of many species' genomes and transcriptomes. For instance, the moss *Physcomitrella patens*, which shares a common ancestor with flowering

plants about 400 million years ago, has a similar suite of biosynthetic and signalling genes to the angiosperm *Arabidopsis*^{6,7}. Moreover, ethylene affects *P. patens*' growth, stimulating growth of peripheral filaments and reducing growth in central area of the moss. Thus ethylene appears to operate similarly throughout land plants separated by 400 million years of evolution. Does the plant ethylene hormone system pre-date the advent of land plants? A recent study demonstrates that it extends back to the charophytes, a division of freshwater green algae, but is lacking in marine green algae⁷. Furthermore ethylene can stimulate cell elongation in the charophytes. Ethylene also regulates cell elongation in flowering plants, for instance inhibiting cell elongation in dark-grown *Arabidopsis* seedlings (Figure 1), but stimulating cell elongation in deep-water rice, suggesting a remarkable conservation of function for ethylene between green algae and flowering plants. The demonstration that the ethylene hormone system exists in charophytes pushes the conservation of this system back to more than 450 million years, when plant colonization of land occurred. Additionally, the use of ethylene as a hormonal signal in the freshwater green algae may have played a role in land colonization, potentially facilitating adaptive responses to various stresses as ethylene often serves as a plant stress hormone.

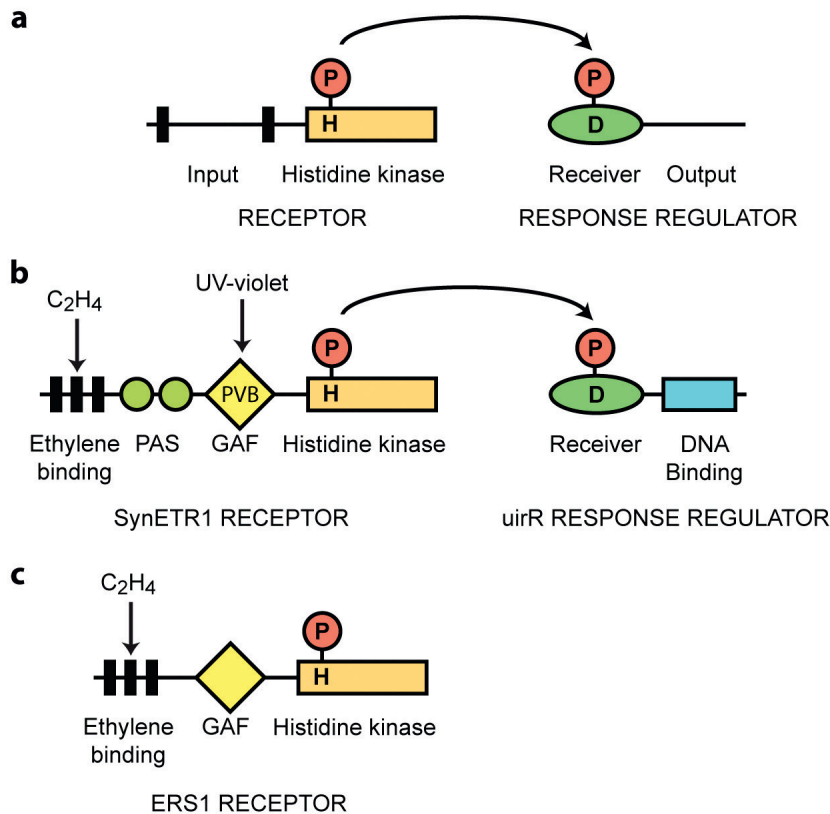


Figure 2. Two-component signalling systems. (a) The canonical two-component system of bacteria incorporates a receptor with histidine kinase activity and a response regulator. Binding of ligand to the receptor regulates phosphorylation on a histidine residue and subsequent transfer to an aspartic acid residue of the response regulator. Black bars indicate transmembrane domains. (b) The ethylene- and light-sensing two-component system of the cyanobacterium *Synechocystis*. The receptor SynEtr1 has an ethylene-binding domain found in its transmembrane region, a light-sensing domain (PVB chromophore attached to GAF domain) and a histidine kinase domain. The response regulator uirR is a transcription factor operating downstream of SynEtr1. (c) Features of the plant ethylene receptor ERS1 of *Arabidopsis*. The receptor has an ethylene-binding domain, a GAF domain and a histidine kinase domain. Note that the GAF domain does not have a chromophore attached and so does not respond to light.

Some plants have lost the ability to use ethylene as a signal

On the basis of the variety of ethylene-regulated processes found in plants, it might seem essential for viability. There is evidence, however, that some plant lineages have lost the ability to use ethylene as a signal⁸. Genome sequencing of eelgrass revealed that the plant lacked genes for ethylene biosynthesis, perception and most of the signalling pathway⁹. Eelgrass is an aquatic plant found in coastal ecosystems around the northern hemisphere. It seems ironic that a signal with an aquatic origin (i.e. the receptors from cyanobacteria and the signalling system from green algae) has been lost in an ocean-living flowering plant. Ethylene, however, may be under a negative selection force in such submerged environments. A gaseous hormone can be problematic as a signalling molecule under water in a multicellular organism because its diffusion efficiency is lower than in air. As a result, the ethylene concentration can build up and negatively affect growth akin to the terrestrial plants exposed to illuminating gas. Indeed, many non-aquatic plants take advantage of this property of ethylene as a signal indicative of submergence, a stress combatted in such ways as the formation of aerenchyma (hollow air cells), elongation to grow out of the submergence zone, or going metabolically quiescent until oxygen levels return to atmospheric levels. Exploiting these ethylene-

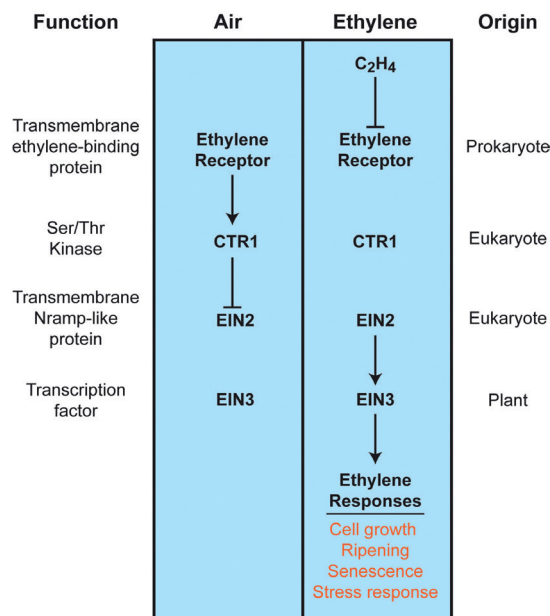
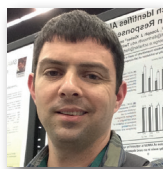


Figure 3. The ethylene signal transduction pathway of plants. The pathway incorporates negative and positive regulatory elements of disparate evolutionary origin. In the absence of ethylene (in air), the serine/threonine kinase CTR1 phosphorylates EIN2 to suppress its activity and prevent an ethylene response. Binding of ethylene to the receptors serves to inactivate CTR1, resulting in activation of EIN2 and the induction of the ethylene response. See Shakeel et al.⁵ for more details.

dependent submergence responses in rice is facilitating the development of new flood-resistant varieties¹⁰. In the case of eelgrass, it is still unknown whether another hormone pathway or an alternative input has taken over ethylene's role to regulate its typical downstream responses. The example of eelgrass and a few other aquatic/semi-aquatic plants demonstrates that ethylene is not essential for plant life under all conditions.

A simple molecule shaping life and culture

There is still much to learn about ethylene and how this simple molecule is integrated into bacterial and plant life. Whether the ancient bacterial ethylene receptors respond mainly to ethylene or other hydrocarbons is still unknown. The precise mechanisms by which ethylene binding to its receptor alters the receptor's activity is not fully understood, nor are some of the downstream regulatory mechanisms. Ethylene signalling and biosynthesis components also interact with other hormone pathways in complex ways that can vary depending on the tissue and plant observed. For such a simple molecule, ethylene's mechanisms are complex and its impact wide. From ancient waters to wounding unripe figs to an urban pollutant affecting plant life to flood-resistant strains of rice, ethylene has had, and continues to have, a profound impact on the biological and human world. ■



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