

Akt-pathway in mammalian cells [10] (Figure 1C). We therefore speculate that higher CKs levels due to aberrant regulation of LOG enzyme may spoil bacterial cells; however, moderate level of CKs may give benefit to the pathogen against animal cells (Figure 1C).

Transkingdom Communication via CKs: Myth or Fact

The ubiquitous distribution of CKs in biosphere [7] is due to their enlarged production by plants [1], plant and animal associated microbes [2,4,5] as well as some insect species [7]. This designates this previously assumed a purely plant specific growth regulator as potent candidate molecules for interactions among various forms of life. Due to their regulatory effects on growth and defense, CKs have already been assumed as core modulators of biological interactions such as plant-pathogen interaction [3], plant-herbivore interaction as well as plant-insect-pathogen tripartite interaction [7] and now animal-pathogen interaction [5] with broader ecological implications. The ability of LOG-domain containing microbial pathogens to cause infection in a host range spanning plants [2,4], mammals [5], amphibians and fish [6], underscores the importance of CKs in crosskingdom microbial pathogenesis. The presence of LOG enzyme homologues in many other important human pathogens such as *Staphylococcus aureus* as well as *Bordetella spp.* [5] are further candidate pathogens that might also secrete CKs (Figure 1A). These recent trends in CKs research provide sufficient base to presume CK being an agent of crosskingdom organismal communication.

However, there are fundamental discrepancies in understanding the signaling cellular circuitry of CK beyond the plant kingdom. Even in plants, many more pertinent questions concerning the crosstalk between CKs and immune pathways are yet to be answered [3]. The report on the secretion of a plant hormone CKs by an

obligate human pathogen [5] prompted exiting questions concerning the functional role of CK in mammalian cells. Quite astonishing is the lack of understanding about the mechanism of the perception of the signal of CK by an animal system while among higher eukaryotes the TCS is found only in plants [1]. Although prokaryotic cells harbor TCS as the sensor pathways to interact with their immediate environment but their perception of CKs is still a mystery. Nevertheless, better understanding of CKs as an agent of crosskingdom communication will help to manipulate biological interactions for sustainable benefits such as crop production, yield protection against microbial pathogens and noxious herbivores as well the targeting of animal pathogens.

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Spotlight Plant Life without Ethylene

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We propose that the ability to synthesize ethylene was selectively lost in evolution when the ancestors of fully aquatic higher plants lost their terrestrial lifestyle. We suggest that there has been negative selection on ethylene in these submerged species because it might interfere with growth in permanently deluged environments.

Evolution of ethylene as a plant hormone

Ethylene is a gaseous plant hormone with a plethora of effects on growth and development, including seed germination, plant growth, pathogen resistance, fruit ripening, organ abscission and senescence [1]. In higher plants, ethylene is synthesized via the methionine- and 1-aminocyclopropane-carboxylic acid (ACC) -based Yang cycle. The rate limiting enzymes are ACC synthase (ACS) and ACC oxidase (ACO) whose regulation depends on environmental and endogenous signals (Figure 1A). Mostly this operates via regulation of the expression of ACS, but sometimes also via ACO (e.g., when O₂ levels are limiting) [2]. Interestingly, many production, perception and signaling mutants have mild phenotypes suggesting that ethylene is a modulator rather than a driver of development

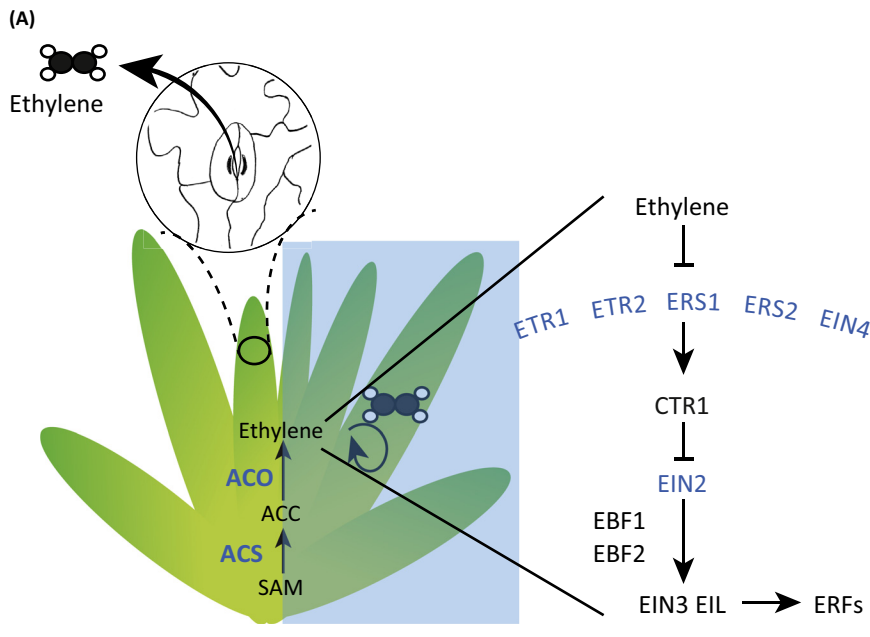


Figure 1. Biosynthesis and Evolution of Ethylene. (A) Plants synthesize ethylene from S-adenosylmethionine (SAM) using two enzymes that synthesize (ACC synthase, ACS) and subsequently oxidize (ACC oxidase, ACO) 1-aminocyclopropane-carboxylic acid (ACC). Ethylene gas can freely diffuse to the atmosphere through stomata, unless full submergence (right half of the plant) prevents this diffusion leading to considerable accumulation. Ethylene is sensed through a set of ethylene receptors (ETR1, ETR2, ERS1, ERS2, EIN4) that relay the signal via a number of intermediates to the ERF family of transcription factors. Depicted in blue are the ethylene biosynthesis and signaling components that are absent in *Zostera muelleri*. (B) Ethylene has evolved as a plant hormone 450 million years ago during the evolution of the common aquatic ancestor of land plants: the charophyte green algae. The genera mentioned in this spotlight article ordered per phylum of the kingdom Plantae. The time scale in million years ago (mya) indicates their origin.

[3]. However, the constitutive ethylene response mutant *ctr1* has a very strong growth phenotype [4], indicating that ethylene signaling beyond natural thresholds severely impacts plant development.

Ethylene has evolved as a plant hormone 450 million years ago during the evolution of the common aquatic ancestor of land plants: the charophyte green algae. The closer the charophyte lineages (e.g.,

Spirogyra, *Coleochaete*) are related to land plants, the more ethylene-related homologues could be identified [5], indicating that ethylene production and signaling developed during the evolution of the charophytes (Figure 1B). Mosses and gymnosperms have a strong conservation of ethylene biosynthesis, perception and signaling components when compared to *Arabidopsis* (*Arabidopsis thaliana*) [5]. Interestingly, many liverworts, mosses, ferns, lycopods and horsetails produce ethylene via an ACC independent pathway, suggesting that ACC dependency developed relatively late in land plant evolution [6]. However, *Spirogyra pratensis*, a late charophyte, evolutionarily older than mosses, did show an increase in ethylene production when provided with ACC, indicating some ACC oxidase activity [5].

Role of ethylene during submergence of terrestrial plants

Although plant life on land has an aquatic origin, many extant water plants with a (semi)-submerged life style switched back to an aquatic life style [7]. Interestingly, a very early representative of angiosperm evolution, *Montsechia vidalii*, had a fully aquatic life style [8].

It is generally accepted that the balance between ethylene production and outward diffusion determines endogenous ethylene concentrations, since there are no described pathways that degrade or inactivate ethylene. Outward diffusion of ethylene is severely hampered under water, resulting in rapid tissue accumulation. Submergence-entrapped ethylene can stimulate cell elongation and this principle is exploited by certain terrestrial angiosperms and aquatic plants with floating leaves (e.g., water lily) to escape from flooding. Flood-adapted terrestrial plants use the ethylene signal to sense submergence in a quick and reliable way and the accumulated ethylene initiates many flood-adaptive responses including accelerated shoot elongation, hyponasty, aerenchyma formation, formation of adventitious roots and growth retardation [9].

Interestingly, the moss *Physcomitrella patens* [10] and the charophyte green alga *Spirogyra pratensis* [5] also demonstrated elongation responses when completely submerged and exposed to elevated ethylene levels suggesting that ethylene-driven elongation as an adaptation to submerged environments developed early in plant evolution.

Studies in deepwater rice and *Rumex* have revealed that elongation initiated by ethylene accumulation involves the down regulation of abscisic acid (ABA) and the stimulated action of gibberellin (GA) and indole acetic acid (IAA). Furthermore, proteins associated with light signaling responses such as the bHLH proteins PHYTOCHROME INTERACTING FACTOR (PIF) and KIDARI and the rice ethylene response factor (ERF) transcription factors SNORKEL1 and 2 are important in the signaling network that leads to fast underwater elongation [9]. The aim of this response is obvious: escape from the submergence in water to allow unhindered inward diffusion of air to aerate organs that are still under water. This elongation response is, however, costly and therefore predominantly restricted to species inhabiting environments with relatively shallow water allowing successful emergence. A completely antithetical ethylene-mediated growth response is observed in some flood tolerant rice varieties and in the wild plant *Rumex acetosa*. These plants dampen growth and thereby conserve carbohydrates to survive longer-lasting floods. In rice this ethylene-mediated response is also regulated by an ERF, called SUB1A. One of the actions of this protein is to repress the action of GA and therefore plant growth [9].

Several permanently submerged species lack components of the ethylene biosynthesis and/or signaling pathway

A comparative genomics approach using unassembled whole-genome shotgun sequence data was used to describe gene conservation and loss in the monocot *Zostera muelleri* [11], a sea grass species

with a fully submerged, marine life style. This genome-wide survey revealed that *Z. muelleri* lacked orthologues of ACS and ACO, of the ethylene receptor-encoding genes, and of the gene encoding the ethylene signal transducer protein ETHYLENE INSENSITIVE2 (EIN2). Interestingly, genes encoding the master transcription factors relaying the ethylene signal such as *EIN3*, *EIN3-like1* (*EIL1*), and *EIL3* were present in the *Zostera* genome. EIN3 transcription factors are integration nodes for various plant hormones and metabolites and this possibly explains their maintenance. A comparison of EST data revealed also a complete lack of genes coding for ethylene biosynthesis, perception and signaling in two other related sea grasses: *Z. marina* and *Z. noltii*. Interestingly, *Spirodela polyrhiza* (duckweed), a monocot with a floating life style from the same order of Alismatales as *Zostera*, does have ACS, ACO, ethylene receptors, *CONSTITUTIVE TRIPLE RESPONSE1* (*CTR1*), and *EIN2* orthologues. This was also true for some terrestrial species belonging to the order of Alismatales [11].

Approximately 20 years ago Summers *et al.* [12] observed that young shoots of the freshwater monocot *Potamogeton pectinatus* were unable to produce ethylene. Although no sequencing efforts were made at that time, it was found that *P. pectinatus* does accumulate ACC, suggesting ACS activity, but is unable to convert it to ethylene indicating that the ACO protein(s) are either not present or not active. The presence of proteins involved in ethylene signaling was not studied for *P. pectinatus*, although its response to exogenous ethylene suggests that this pathway is intact [13].

The similarity between the *Zostera* and *Potamogeton* species is that both are angiosperms with an aquatic life style. We speculate that angiosperm species with a submerged life style may not require ethylene for growth and development. In fact, the apparent absence of ethylene production, perception and signaling

might indicate that there has been negative selection on ethylene in these submerged species. The absence of the complete ethylene biosynthesis and signal transduction pathway in *Zostera* further suggests that loss of one essential component in ethylene biosynthesis or signaling relaxed the selective pressure of all genes involved in this pathway [11].

Why have *Zostera* species and *Potamogeton pectinatus* lost ethylene production and/or signaling?

As mentioned before, ethylene accumulates to relatively high levels in submerged plants, due to limited outward diffusion. Plant responses to ethylene are typically biphasic [3], with often severe growth inhibition at high concentrations. However, frequently submerged, flood-adapted terrestrial plants respond only with growth modifications to relatively high endogenous ethylene levels ($1\text{--}10\ \mu\text{l}^{-1}$), typical for temporary submerged conditions, indicating that in fact they are very insensitive to ethylene.

Aquatic plants with a completely submerged life style including *Zostera* and *Potamogeton* would also accumulate high levels of ethylene if they had the capacity to produce it. However, unlike temporarily flooded plants, these high ethylene levels would persist in these permanently submerged aquatic plants. Consequently, accumulated ethylene at high concentrations could have a negative impact on the physiology and growth of these species, similar to what is observed in constitutive ethylene signaling mutants in *Arabidopsis* (i.e., *ctr1*; [4]). Based on this we hypothesize that in these plants ethylene biosynthesis and/or signaling was selectively disfavored in the transition of some angiosperm plants from a terrestrial to an aquatic life style.

An interesting question then remains: why can the fully aquatic Charophytic algae *Spirogyra pratensis* still grow very well, whilst producing ethylene in an ACC

dependent way? Part of the answer may be that alternative ways exist to regulate endogenous ethylene. In addition, it appears that *Spirogyra* does not show a growth inhibition response at very high ethylene concentrations, and thus is unlikely to be inhibited by excessive ethylene [5].

The lack of ethylene biosynthesis and/or signaling in Angiosperm plants with a submerged life style is only known for two genera, and the absence of ethylene biosynthesis and responses in the *Zostera* species still needs physiological and biochemical confirmation, stressing the need for further systematic studies into the ethylene biology of permanently submerged plants.

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