[Plant Signaling & Behavior 3:10, 761-763; October 2008]; ©2008 Landes Bioscience

Mini-Review

Ethylene signaling regulates salt stress response

An overview

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Abbreviations: CTR1, constitutive triple response 1; EIN2/3/4, ethylene-insensitive 2/3/4; ETR1/2, ethylene response 1/2; ERS1/2, ethylene response sensor 1/2; NTHK1, *Nicotiana tabacum* histidine kinase 1; ERF, ethylene-responsive factor; ACC, 1-aminocyclopropane-l-carboxylic acid

Key words: ethylene, ethylene signaling, ethylene receptor, salt stress

Ethylene has long been regarded as a stress-related hormone, but only recently the link between ethylene signaling pathway and salt stress was primarily established. Ethylene signaling modulates salt response at different levels, including membrane receptors, components in cytoplasm, and nuclear transcription factors in the pathway. However the relevant mechanism is still unclear. In this paper, we described how ethylene signaling pathway regulates salt stress response and discussed the challenges of ethylene and receptor signaling in salt response regulation.

Introduction

Salinity is one of the most severe stresses among the abiotic stresses and affects crop production of about 23% of cultivated land.[1] Plant responses to salt stress is regulated by multiple internal and external factors.[1,2] Ethylene, as a gaseous plant hormone, is also involved in plant stress responses, in addition to its roles in germination, fruit ripening, organ abscission, pathogen response and senescence etc.[3-5] In the past two-decades, the linear signal pathway of ethylene has been established based on the identification of ethylene-response mutants in Arabidopsis.[3,5] Ethylene is perceived by a family of receptors with sequence similarity of bacterial two-component histidine kinase. Downstream of the receptor is CTR1, which is also a negative regulator in the pathway. EIN2 and EIN3 are positive regulators of ethylene response, acting as downstream components of CTR1, and EIN3 mediates transcriptional cascade of ethylene-regulated genes.[3-5] Although ethylene is generally believed as a stress-hormone and ethylene signaling regulates multiple stress responses, it is not clear what specific roles the different components of ethylene signaling pathway play in salt stress responses. Only recently, the roles of ethylene signaling in salt stress response were gradually disclosed.[6-9] Here, we reviewed and discussed the recent advances about the regulation of plant responses to salt stress by ethylene signaling pathway.

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Submitted: 03/19/08; Accepted: 03/19/08

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/5934

Ethylene Receptor Signaling and Salt Stress Response

Ethylene receptor is divided into two groups according to their sequence similarity and structure characteristics in different plant species. In Arabidopsis, ETR1 and ERS1 belong to the group I subfamily, while ETR2, EIN4, and ERS2 are subfamily II receptors. The expression of *ETR1* gene seems to be downregulated by salt and osmotic stress at both transcription and protein levels in Arabidopsis.[10] However, many ethylene receptor genes can be induced by multiple stresses.[11,12] The gain-of-function mutant *etr1-1*, with ethylene insensitivity, showed increased sensitivity to salt stress during germination and early seedling development.[6,9,13] On the contrary, the strong loss-of-function mutant *etr1-7* showed increased tolerance to salt stress.[13] The gain-of-function mutants *ein4-1* and *etr2-1*, and Arabidopsis plants with overexpression of dominant mutation of *ERS2* (mutation in ethylene binding site in transmembrane domain) also exhibited enhanced sensitivity to salt stress compared to wild type (Li ZG, Chen SY and Zhang JS, unpublished data).[6] These analyses indicate that ethylene receptors regulate salt-sensitive response. Studies on a subfamily II ethylene receptor NTHK1 from tobacco demonstrated the similar conclusions. Transgenic Arabidopsis and tobacco plants overexpressing the *NTHK1* showed sensitivity to salt stress compared to wild type plants.[6,8,9] NTHK1 contains four transmembrane domains, a GAF domain, a kinase domain and a receiver domain. Biochemical studies have revealed its serine/threonine kinase activity.[14] The role of different domains of NTHK1 was analyzed in transgenic Arabidopsis plants and it is found that the kinase domain of NTHK1 is required for salt-sensitive epinasty phenotype while the receiver domain is not required for this response. Further mutation of the N-box in kinase domain abolished the NTHK1 kinase activity and resulted in the loss of salt sensitivity in transgenic plants, suggesting that the NTHK1 Ser/Thr kinase activity is essential for the salt sensitive response (Chen T, Liu J, Chen SY and Zhang JS, unpublished data).

It is interesting to find that ACC (an ethylene precursor) suppresses the salt sensitivity conferred by NTHK1 in transgenic Arabidopsis plants, suggesting that ethylene is required for counteraction of receptor function to improve salt tolerance. Interestingly, the*NTHK1* mRNAs accumulate in both transgenic. Arabidopsis and tobacco plants under salt stress treatment and this accumulation does not require de novo protein synthesis,[6,8,9] suggesting that the regulation of the *NTHK1* mRNA level follows a posttranscriptional mechanism. Further analysis revealed that the salt-responsive element was located in the transmembrane-coding region but not in the promoter region.[9] This phenomenon may indicate a special mechanism for regulation of ethylene receptor genes in response to salt stress.

Ethylene receptors may function in stress response through regulation of salt-responsive gene expressions. Overexpression of the tobacco ethylene receptor gene *NTHK1* in Arabidopsis plants promoted expression of *AtERF4, Cor6.6, rd17, RD21A,*and *VSP2*, but inhibited expression of *BBC1, Lea* and *AtNAC2* genes.[6,9,15] In *etr1-1* and *ein4-1* gain-of-function mutants, the *Cor6.6* was also consistently enhanced. In tobacco plants overexpressing *NTHK1*, salt-induction of the *NtACS1* was suppressed whereas the NTHK1 appears to promote early induction of the *NtACO3* gene. Salt-induction of the *NtERF1* and *NtERF4* was also stronger in *NTHK1*-transgenic tobacco plants compared to wild type plants.[9] Altered downstream gene expressions may contribute to the salt stress response conferred by overexpression or gain-of-function of ethylene receptors.

Arabidopsis subfamily I receptors ETR1 and ERS1 interact with CTR1 and regulate downstream response. However, subfamily II receptors (ETR2, EIN4, and ERS2) appear to have only very weak interactions with CTR1.[16,17] Studies also have shown that EIN3 protein turnover remains responsive to ethylene in *ctr1* loss-of-function mutant but not in ETR1 loss-of-function mutant *etr1-7*, and the loss-of-function *ctr1* mutant still have a little ethylene response. Moreover, the quadruple mutant of ethylene receptors of Arabidopsis has more severe phenotype than null mutant *ctr1*. All these facts indicate that another by-pass signaling pathway independent of CTR1 but acting downstream of ethylene receptors may exist. Using a CytoTrap yeast two-hybrid system, we have identified a novel NTHK1-interacting protein (Nip) which associates with tobacco ethylene receptor NTHK1 to mediate salt and other stress responses in transgenic tobacco plant (Cao YR, Chen SY and Zhang JS, unpublished data). Further investigation should shed light on the role of this protein in mediating ethylene receptor signaling and regulation of stress responses.

Other Components of Ethylene Signaling and Salt Stress Response

The functions of other components of ethylene signaling in salt stress response were also investigated. The *ctr1-1* mutant exhibited increased salt tolerance, and germination rate and post-germination development of *ctr1-1* are more tolerance under salt and osmotic treatments, especially under high concentration of salt stress,[7,18] indicating that CTR1 acts as negative regulator to salt stress. EIN2 is a central component of the ethylene signaling transduction pathway in plants, and its null mutant *ein2* has complete insensitivity to ethylene at the morphological, physiological, and molecular levels in Arabidopsis.[19] Under salt stress, the *ein2-1* mutant is severely affected in both seedling growth and in seed germination process, suggesting that EIN2 is required for salt stress tolerance.[6,7] It should be mentioned that because ethylene signaling affects seed germination and ethylene-responsive mutant seeds often have enhanced or delayed germination under normal condition, explanation for the roles of ethylene signaling components in germination process under salt and other stresses need to be cautious. The EIN2 may also act as a cross-talk point for multiple signaling pathways involving hormones and stesses.[7,19,20] EIN3 is an important component downstream of EIN2 involved in ethylene signaling. As a plant-specific transcription factor, EIN3 regulates multiple ethylene-related gene expression, and binds directly to the promoter region of ERF1 (ethylene-responsive factor 1), and ERF1 then evoke its downstream genes expression. EIN3 degradation is controlled by two F-box proteins EBF1 and EBF2.[21,22] The *ein3-1* mutants (lacking EIN3) exhibited reduced salt tolerance at higher salt concentration but not at lower concentration and that *ebf1-1ebf2-1* mutants exhibited increased salt tolerance.[6,18] The increased salt tolerance of *ebf1-1 ebf2-1* mutants was abolished by lack of EIN3 in *ein3-1 ebf1-1 ebf2-1* mutant plants.[18] Overexpression of several *ERF* genes enhanced salt tolerance in transgenic lines.[23,24] However, it should be noted that connection of these *ERF* genes into ethylene signaling needs to be confirmed and it is possible that some of these *ERF* genes is not related to ethylene signaling. AtNAC2, another transcription factor regulates lateral roots development and salt response in Arabidopsis, whose gene expression is induced by salt treatment through ethylene signaling and auxin signaling.[15] Recently, a novel component MKK9 was found to act downstream of CTR1 to directly modulate EIN3 function in ethylene signaling.[25] The *mkk9* mutant displayed a broad spectrum of ethylene-insensitive phenotype, including salt stress sensitivity.[25]

Conclusion and Perspective

Plants have evolved various specific mechanisms to adapt themselves to the changing environment. Ethylene signaling is one of these pathways that plants have adopted for regulation of salt stress responses. However, many puzzles inside in the ethylene signaling pathway remain to be elucidated. For example, since overexpression or gain-of-function of ethylene receptors results in salt sensitivity, is it possible that these receptors function as sensors for stresses in the absence of ethylene? Because salt stress induces ethylene-biosynthesis genes and ethylene production,[6,18] ethylene may then inhibit its receptors, suppress salt sensitivity conferred by ethylene receptors and promote ethylene responses including salt tolerance. Negative regulation between ethylene and its receptors has been established.[26] This speculation may explain the fact that salt-stressed *NTHK1*-overexpressing Arabidopsis plants, with salt sensitive phenotype, can recover after growth for a longer period in plates, possibly due to the accumulation of ethylene in plants or in plates (data not shown). We thus propose a model for the role of ethylene signaling in the adaptive response of plants to salt stress (Fig. 1). Salt tolerance or sensitivity may depend on the balance or homeostasis between ethylene and its receptors in a plant. If receptor function or signaling is intensified, the plants will have large rosette, late flowering but show sensitivity to salt stress.[6] If ethylene production or signaling is enhanced, the plants will have small rosette, relatively early flowering but more tolerance to salt stress. Neither of these two extreme situations is beneficial for plant life cycle. In the former case, the plants may have a good vegetative stage but has a risk of producing no or

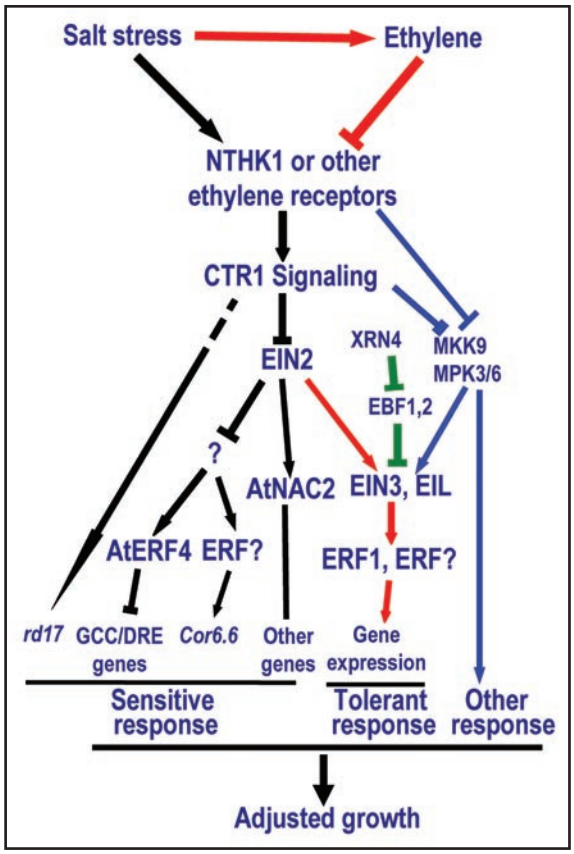

Figure 1. Ethylene signaling and salt stress response. Black arrows and lines indicate active receptor signaling, which leads to temporary sensitive response under salt stress through suppression of AtNAC2 and activation of AtERF4. Red arrows and lines indicate active ethylene signaling, which inhibits receptor signaling and leads to tolerant response under salt stress. Green lines and bars indicate the pathway regulating the EIN3 stability. Blue arrows and lines indicate new pathway bypassing EIN2 and regulating ethylene response and salt stress response.[25]

less seeds. In the latter case, the plants may have only very limited energy and resources for production of seeds due to limited vegetative growth. The plants need to make adjustments at various levels for ethylene and receptor signaling to avoid the two above situations and finally can complete the life cycle smoothly or survive safely. The model needs to be tested with further studies.

Signal transduction from ethylene receptors to the downstream components also requires further attention especially at the biochemical level. The difference in the signal output of ethylene receptors in the presence or absence of ethylene is a further challenge for scientists. As a central integrative component of the pathway, EIN2 is a large protein (about 140 KDa) with 12 transmembrane domains in the middle part.[19] How this protein accepts signal and transduces signals to other components remains to be an open question. From ethylene receptors to EIN2, new components may be incorporated in addition to CTR1. Some responses exerted by receptors may even not require EIN2. All these studies should be conducted in relation to salt or other stress responses and will certainly advance our understanding of the roles of ethylene signaling pathway in plant response to salt stress.

Acknowledgements

This work was supported by the key project of National Science Foundation of China (90717005) and the National Basic Research Program of China (2006CB100102). We apologize if some references are not cited due to space limits.

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