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Review

Progress in the participation of Ca^{2+}–calmodulin in heat shock signal transduction

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Abstract

A novel heat shock (HS) signal transduction pathway in plants for the participation of Ca^{2+}–calmodulin (CaM) in HS signal transduction was identified. HS induces a rapid increase in intracellular free calcium ion levels ($[Ca^{2+}]_i$), and the involvement of phospholipase C-inositol 1,4,5-trisphosphate is one of the factors leading to elevation in $[Ca^{2+}]_i$ induced by HS. HS also increases the expression of the CaM gene and the accumulation of the CaM protein. The CaM isoform, AtCaM3, in *Arabidopsis* is a key member in the HS signal transduction pathway. AtCaM3 regulates the activity of CaM-binding protein kinase (AtCBK3) or protein phosphatase (AtPP7), promoting the activation of the HS transcription factor, AtHSFA1a, by phosphorylation/dephosphorylation and the expression of heat shock protein genes, then improving heat tolerance in plants.

Keywords: Ca^{2+}; Calmodulin; Protein kinase; Heat shock transcription factor; Signal transduction

1. Introduction

Global warming due to human activities has become a common understanding of meteorologists in the world. The Intergovernmental Panel on Climate Change (IPCC) sent out a strong warning on climate crisis due to global warming in the IPCC Fourth Assessment Report. This report pointed out that warming of the climate system is unequivocal; the increase in the global average air temperature will reach 1.1–6.4 °C during the 21st century. It is predicted that the production of the main cereal crops will decrease by 5–10% during the next 20 years due to global warming. The study of thermotolerance in plants becomes important and imperative in this situation. The study of critical genes controlling thermotolerance in plants is the basis for understanding the mechanism of adaptation to elevated temperature. How cells perceive and transduce outside stimuli, the changes in physiological reaction and gene expression caused by the stimuli, and the molecular pathway of modulation and transduction are main interest points in cell signal transduction. The study of the heat shock signal transduction pathway is one of the ways by which we are able to find genes related to thermotolerance in plants. The mechanism by which plants withstand environmental stresses remains largely unknown. The investigation in this field is currently at the preliminary stage.

Plants are both sessile and poikilothermic; they can neither move to avoid environmental stresses nor adjust their core temperature to withstand them. As a result, they have to evolve an elaborate stress response network and a wide array of mechanisms for adapting to stressful

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environments. This may be the crux of survival for plants under changing environments. One of the best characterized responses to environmental stresses is the induction of heat shock proteins (Hsps). The heat shock (HS) response has been observed in every organism studied to date. Hsps function as molecular chaperones, which are essential for cellular processes including protein folding, subcellular localization, assembly and signal transductions. Under stress conditions, the synthesis of molecular chaperones allows cellular proteins to avoid and/or recover from stress-induced protein aggregation [1–4]. The functional analysis for individual Hsp has been documented. The members of the Hsp100 family play a critical role in heat tolerance [5,6]. The role of small Hsps in elevating thermotolerance in tobacco (*Nicotiana plumbaginifolia*) seedlings has been reported [7,8]. Charng et al. [9] reported that a novel heat-stress-associated 32-kD protein (Hsa32) accumulated in *Arabidopsis thaliana* seedlings after HS, which is required not for induction but rather maintenance of acquired thermotolerance. The results in our laboratory showed that Hsp genes *atDjA2* and *atDjA3* play roles in improving thermotolerance [10]. In addition to Hsps, heat shock transcription factors (HSF), which mediate the transcriptional activation of Hsp genes, have a unique function of increasing thermotolerance in *Arabidopsis* and tomato (*Lycopersicon esculentum*). Among at least 17 members in the tomato HSF family, LpHsfA1 has a unique role as master regulator of thermotolerance [11]. The suppression of *AtHsfA1a* activity impaired basal and acquired thermotolerance in *Arabidopsis* [12]. Li et al. [13] provided the proof for regulation of *AtHsfA2* on expression of heat stress genes and thermotolerance in *Arabidopsis*. Charng et al. reported that the *AtHsfA2* knockout mutant showed more sensitivity to severe heat stress than the wild type [14]. The role of *AtHsfA3* regulated by upstream transcription factor DREB2A in improving thermotolerance in *Arabidopsis* has been documented recently [15,16].

However, the pathways by which HS signals are perceived and transduced to activate Hsp gene expression and induce thermotolerance remain unknown. Only a few models have been offered previously. Ananthan et al. [17] proposed that the accumulation of stress-denatured proteins could be the signal for increased expression of Hsp genes. The involvement of membrane fluidity and mitogen-activated protein kinases (MAPKs) in HS signal transduction was proposed [18]. Recent evidence shows that multiple signaling molecules, including H_2O_2, ethylene, abscisic acid (ABA) and salicylic acid (SA), are involved in HS response. The generation of reactive oxygen species (ROS) can induce Hsp synthesis, suggesting an intimate connection between HS response and oxidative stress [19–22]. Our results indicated that HS induced a rapid increase in intracellular free calcium ion levels ($[Ca^{2+}]_i$) of wheat (*Triticum aestivum* L.) seedlings and *Arabidopsis* suspension cells. The increase in $[Ca^{2+}]_i$ regulates expression of Hsp genes and synthesis of Hsps through the following signaling molecules: calmodulin (CaM) → CaM-binding protein kinase or CaM-binding protein phosphatase → the increase in binding activity of HSF to the heat shock element (HSE) → induction of Hsp genes → accumulation of Hsps, then improving heat tolerance in plants. Based on our findings, we propose a pathway for the participation of Ca^{2+}–CaM in HS signal transduction. Herein, we discuss the signal molecules involved in the novel HS signal transduction pathway, combining our study with the research progress in the field worldwide.

2. Calcium initial response of heat shock signal transduction

Ca^{2+} has firmly been established as a primary intracellular second messenger in plants and is widely employed by eukaryotic organisms to regulate a variety of cellular processes directly or indirectly [23–26]. It was reported that HS induced a large increase in $[Ca^{2+}]_i$ in *Drosophila melanogaster*, Chinese hamster and HeLa cells [27]. Heat shock also induced a 4-fold increase in the $[Ca^{2+}]_i$ in the protoplast of pea (*Pisum sativum*) leaves [28]. Gong et al. [29] observed that HS caused a rapid and transient increase in $[Ca^{2+}]_i$ in tobacco transformed with the Ca^{2+}-sensitive, luminescent protein aequorin. We also observed that the initiation of this $[Ca^{2+}]_i$ increase occurred within 1 min of HS in wheat tissue. After 4 min of HS, the $[Ca^{2+}]_i$ reached a maximum 3-fold increase [30]. In suspension-cultured *Arabidopsis* cells expressing aequorin, $[Ca^{2+}]_i$ increased rapidly after HS and reached a maximum after 10 min of HS [31]. Further results demonstrated that the expression of Hsp genes was up-regulated by the addition of $CaCl_2$ and down-regulated by the calcium ion chelator EGTA, the calcium ion channel blockers $LaCl_3$ and verapamil, during HS at 37 °C. Moreover, Ca^{2+} is also involved in the synthesis of Hsps and HS-induced thermotolerance in wheat [30,32,33]. The rapid elevation in $[Ca^{2+}]_i$ caused by HS indicates that the change in $[Ca^{2+}]_i$ is an initial response of HS signal transduction. However, the molecular mechanism for rapid elevation of $[Ca^{2+}]_i$ induced by HS is not well understood.

Ca^{2+} ion channel genes in response to heat and cold sensors have been cloned from the dorsal root or trigeminal ganglia in mammals, which belong to the mammalian transient receptor potential (TRP) family of ion channels. Cold and thermal stimuli can be perceived by TRP, activating and turning on the TRP ion channel, allowing Ca^{2+} into neurons, resulting in a series of neural responses [34–36]. However, there has been no sequence data so far that could confirm the existence of the homologue of the TRP ion channel family in plants.

Major routes for influx of Ca^{2+} into cytoplasm from extracellular sources and intracellular Ca^{2+} pools are Ca^{2+} channels in the plasma membrane and endomembrane systems. The mechanisms by which Ca^{2+} channels are regulated are quite complicated [37]. The phosphoinositide-signaling pathway (PLC-IP_3) is one of the probable pathways by which Ca^{2+} entry into the cytoplasm from intracellular Ca^{2+} pools, causes the elevation in $[Ca^{2+}]_i$.