综 述

Role of Small GTPase in Plant Disease Resistance: Rice as A Model

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Abstract Small GTPases are low molecular weight GTP-binding proteins with molecular weight in the range of 20~30 kDa. They are "molecular switches" involved in a variety of signal transduction pathways that regulate diverse cellular functions. Recently, studies from model plant rice have shown that the GTPase Rho family plays an essential role in the regulation of disease resistance in rice (*Oryza sativa*). In this paper, we give an up-to-date review of the progress on mechanisms underlying a plant-specific Rho-type GTPase mediated immune responses in rice.

Key words plant disease resistance; small GTPases; rice

1 Introduction

Rice (*Oryza sativa*) is an important staple food for over half of the world population. Rice has also become a model system of monocotyledon plants for biological research. For example, the rice-blast pathosystem has been a perfect model for understanding the host-pathogen interactions. With the completion of rice genome sequencing project and the launch of functional genomics study, remarkable progress has been achieved in uncovering the molecular mechanisms underlying rice defense against pathogen infection, especially in dissecting the signaling pathway for induction of immune response. One of the highlighted progresses in this aspect was the identification of the small GTPase OsRac1, a molecular switch which plays an essential role in the regulation of rice immunity. This review is focused on the recent advances in the study of OsRac1 mediated regulation of rice immunity.

2 Plant disease resistance genes and defense response system

Upon the infection of pathogen, plants are capable of initiating two defense lines. The first line of active defense in plants is triggered by invariant microbial epitopes known as pathogen-associated molecular patterns (PAMPs). PAMP-triggered immunity (PTI) is the primary immune response which has evolved to recognize common features of microbial pathogens. With the co-evolution of plants and pathogens, however, pathogens acquired the ability to suppress PTI by delivering effector proteins to the plant cell which block the immunity pathway. In response, plants evolved specific resistance(R) protein alleles for surveillance of the presence of the pathogen effectors and produced effector-triggered immunity(ETI), which constitutes the second line of defense[1,2]. R-protein-mediated defense responses are frequently associated with a type of programmed cell death termed the hypersensitive response (HR). The HR

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Abbreviations: PAMP, pathogen-associated molecular patterns; PTI, PAMP-triggered immunity; ETI, effector-triggered immunity; HR, hypersensitive response; ROS, reactive oxygen species; SAR, systemic acquired resistance; R gene, Resistance gene; Avr gene, Avirulence gene; NBS, nucleotide binding sites; LRR, leucine rich repeats; G proteins, GTP-binding proteins; GAPs, GTPase activating proteins; GEFs, Guanine nucleotide exchange factors; GDI, guanosine nucleotide dissociation inhibitors; CA, constitutively active; DN, dominant negative; SE, sphingolipid elicitor; FRET, fluorescence resonance energy transfer; MAPK, mitogen-activated protein kinase.

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occurs at the infection sites and serves to restrict the growth and spread of pathogens to other parts of the plant. Cells undergoing the HR are often accompanied by producing reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide, hydroxyl radicals and nitrous oxide. ROS act as signaling molecules in plant responses as well as having antimicrobial properties directly. Local HR in turn triggers a long lasting systemic response (systemic acquired resistance, SAR) that primes the plant for resistance against a broad spectrum of pathogens[3,4].

Over the past two decades, dozens of R genes, against many different pathogens, have been isolated from a variety of plants[5,6]. Based on the conserved functional protein domains, these R proteins can be grouped into several superfamilies, the vast majority of them belong to the nucleotide binding sites (NBS) and leucine rich repeats (LRR) kinase superfamilies. R genes encode putative receptors that respond to the products of 'Avr genes' (Avr, avirulence) expressed by the pathogen during infection. Interaction of R protein and Avr protein, directly or indirectly, triggers the defense signaling pathway[5]. Some PAMP receptors have been characterized as well, including the LysM domain-containing receptor-like kinases CERK1[7] and LysM RLK1[8] for chitin signaling and resistance to fungal pathogens, the FLS2 (flagellin-sensitive 2) and EFR (elongation factor Tu receptor) for perception of the bacterial flagellin and elongation factor, respectively, and confer resistance to bacterial pathogens[9]. The identification of these R proteins and PAMP receptors has opened the gateway to reveal the complicated plant defense signaling pathway. However, downstream these receptors and early events what are the relay components and how they activate the signaling cascades which trigger the immune response, are largely unknown.

3 Plant small GTPase Rho, role and its regulation

Small GTP-binding proteins (G proteins) are monomeric G proteins with molecular masses of 20~40 kDa. Small G proteins exist in eukaryotes from yeast to human and constitute a superfamily with at least five families (Ras, Rho, Rab, Sar1/Arf and Ran) including more than 100 members[10]. Small G proteins act as molecular switches and regulate a wide variety of important cell physiological functions including cell proliferation, cytoskeleton organization, intracellular trafficking, and immunity response. The conserved protein domains of small G proteins are guanine nucleotide binding motifs and domains important for interaction with its regulators and effectors. A typical small G-protein is active when bound to GTP and inactive when bound to GDP, which can be then replaced by free GTP. GTP hydrolysis is accelerated by GTPase activating proteins (GAPs), while GTP exchange is catalyzed by Guanine nucleotide exchange factors (GEFs). Activation of a GEF typically activates its cognate G-protein. Guanosine nucleotide dissociation inhibitors (GDI) maintain small GTPases in the inactive state[11]. The working model for small G-protein was described as follows: upon the upstream signal stimulation, the small G-protein changes into active form and leads to the conformational change of the downstream effector-binding region so that this region interacts with the downstream effector(s). In this way, this interaction transduces an upstream signal to a downstream effector(s)[12].

Plants have only four of these five families of small G proteins with lack of the Ras family. Instead, they have a unique subfamily of Rho-family GTPases, called ROPs (Rho-related GTPase from plants). ROPs share a common ancestor with Rho, cdc42 and Rac in animal and yeast, and are also referred to as RACs. It has been proposed that ROPs act as a predominant GTPase switch to control the transmission of extracellular signals in plants, and regulate various plant cellular responses including cytoskeletal organization and dynamics, pollen tube growth and development, and vesicle traffic[13]. In recent years, evidence is accumulating that ROPs play an important role in the regulation of disease resistance[13,14]. Binding of the ligand from the pathogen with the corresponding receptor in plants, either R protein or PAMP receptor, can lead to turn on the ROPs which in turn relay the signal to downstream effectors and result in

the defense response ultimately.

4 Small G protein mediated disease resistance in rice

4.1 OsRac1, a member of the Rho family, plays an essential role in rice defense against pathogen

In the last decade, about 37 R genes against the blast fungus pathogen *Magnaporthe oryzae* and 29 R genes against the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae*(*Xoo*) have been identified, more than 10 of them, including the NBS/LRR domain-containing proteins or receptor-like kinases, have been cloned[15]. It has been shown that the resistance of rice to blast pathogen *M. grisea* is triggered by a physical interaction between the protein products of the host R gene, *Pi-ta*, and the pathogen Avr gene, *AVR-pita*[16]. The rice resistance gene *Xa21* confers resistance against most of the known races of *Xoo*. Xa21 protein contains an extracellular LRR domain, which is proposed to be responsible for specific recognition of a pathogen produced ligand, a single-pass transmembrane domain, and a serine threonine kinase (STK) intracellular domain[17]. This molecular structure of Xa21 represents a unique class of plant disease resistance genes, suggesting a role in cell surface recognition of a pathogen ligand and subsequent activation of an intracellular defense response. Chitin oligosaccharides have been known for a long time as elicitors to induce defense responses in plants through binding with their cognate receptors. A chitin elicitor receptor, CEBiP, was identified from rice, which plays a key role in the perception and transduction of chitin elicitor in the rice cells[18]. These described receptors are thought to transduce an external signal to initiate the immune response. However, the molecular machinery involved in the signal transduction pathway downstream receptor-ligand recognition, has not yet been well defined.

To address this challenging question, some researches focus on the small G protein with the attempt to find clues. In rice, there are total 7 members in ROPs family[19]. The Ko Shimamoto's group found one of them, OsRac1, which is a key player in regulation of rice defense response against pathogen. *OsRac1* encodes a GTPase similar to its homologue in mammals. Transgenic rice expressing constitutively active(CA) OsRac1(OsRac1-G19V), either in leaves or cultured suspension cells, induced ROS production and apoptosis-like cell death. In contrast, transgenic rice expressing the dominant negative (DN) OsRac1 (OsRac1-T24N) blocked ROS production and cell death, indicating that OsRac1 was required for activation of ROS production and was a regulator of cell death in rice[20]. Further experiments demonstrate that transgenic rice lines expressing CA-OsRac1,but not DN-OsRac1, caused HR-like responses, enhanced resistance against blast fungus and bacterial blight, caused increased production of a phytoalexin and altered expression of defense-related genes[21]. OsRac1 was also implicated in the regulation of fungal sphingolipid elicitor (SE) induced rice defense response as well. By using proteomics method, proteins whose expression levels were altered by OsRac1 and/or SE treatment were systematically analyzed. The result showed that 100 proteins were up-regulated by a SE, 87 were also induced by CA-OsRac1[22], suggesting that OsRac1 plays a pivotal role in defense responses induced by elicitor in cultured rice cells. As SE elicitor is a typical PAMP molecular, this finding imply that OsRac1 is an important regulator of rice basal immunity. Taken together, these findings strongly suggest that OsRac1 has a general role in disease resistance of rice.

4.2 Molecular mechanisms of OsRac1 mediated defense response in rice

Following the demonstration of the pivotal role of OsRac1 in rice defense response, dissecting of its signalling pathway, such as seeking of its upstream regulators, downstream effectors and interacted or interconnected partners became the main tasks. Significant progress has been made in finding of the components involved in OsRac1 signaling pathway in recent years.

4.2.1 OsRac1 controls ROS production through regulation of the NADPH oxidase It is now clear that, in response to pathogen infection, ROS are produced by plant cells. ROS may be involved in direct antimicrobial mechanisms or signaling pathway of defense response. The role of ROS was summarized more

recently. In basal resistance, they are linked to formation of barriers. In the hypersensitive response, they may be linked to programmed cell death, and in SAR, they interact with salicylate in signaling. Cell plasma membrane NADPH oxidases and cell wall peroxidases are considered as the two most likely enzymes involved in the generation of ROS[23,24].

In rice, it was found that the small GTPase OsRac1 can regulate the NADPH oxidases catalytic subunit homologue gp91phox, also known as Rboh (for respiratory burst oxidase homolog), and thus lead to the production of ROS. Early research showed that overexpression of the CA-OsRac1 can increase ROS production and enhance resistance to virulent rice fungus and bacterial blight, but the DN-Rac1 causes reduction of ROS levels. Meanwhile, the observed H_2O_2 production was inhibited by diphenylene iodonium (DPI), an inhibitor of the neutrophil NADPH oxidase, indicating that the generation of reactive oxygen species was NADPH-oxidase dependent[20,21,25]. Using the methods of yeast two-hybrid assay, *in vitro* pull-down assay and *in vivo* fluorescence resonance energy transfer (FRET) microscopy, more evidences revealed that CA-OsRac1, but not DN-Rac1, interacted with the N-terminus of rice RBOH(OsRbohB) through two Ca^{2+}-binding EF-hands domains and this interaction was regulated by cytosolic Ca^{2+} concentration. Furthermore, transient co-expression of OsRac1 and OsRbohB in leaves of *Nicotiana benthamiana* enhanced ROS production, suggesting that direct Rac-Rboh interaction may activate NADPH oxidase activity in plants[26]. On the other hand, it was demonstrated that OsRac1 likely potentiates ROS generation through inhibition of the ROS scavenger. The expression of *OsMT2b*, a ROS scavenger gene, was synergically down-regulated by *OsRac1* and rice blast-derived elicitors[27]. These results suggest that OsRac1 plays a dual role as an inducer of ROS production and a suppressor of ROS scavenging. However, there is still a need to finely dissect the overall ROS generating systems and fully understand the control mechanisms of ROS production mediated by the small GTPase.

4.2.2 Enzymes involved in the synthesis of defense related substances and MAPK cascade are effectors of OsRac1 Upon the attack of pathogen, deposition of lignin in the infection site is one of the important defense responses in plants. The lignification strengthens cell walls and interferes with the enzymatic hydrolysis and mechanical penetration of plant tissue by pathogen[28]. OsCCR1, a cinnamoyl-CoA reductase 1 involved in the first committed step of the lignin biosynthesis, has been identified as an effector of OsRac1. Supporting observations include that the expression of *OsCCR1* can be induced by a sphingolipid elicitor and the transgenic rice cell cultures expressing the CA-OsRac1 increased accumulation of lignin. Furthermore, OsCCR1 was screened out as an interactor of OsRac1 when using yeast two-hybrid screening with OsRac1 as an bait. Moreover, OsRac1 was shown to bind OsCCR1 in a GTP-dependent manner and their interaction can led to the enzymatic activation of OsCCR1 *in vitro*[29]. As the production of many other antimicrobial substances such as phytoalexins is also known to be related with the lignin and lignin-related compounds[30], we thereby propose that the enzymes involved in the synthesis of defense related substances could be the downstream effectors of OsRac1, through the activation of them, the defense response was provoked in rice.

In addition, the mitogen-activated protein kinase (MAPK) cascade was shown to be activated during responses to pathogens or elicitors in rice and, more significantly, was regulated by the OsRac1. A rice MAPK gene, *OsMAPK6*, was posttranslationally activated in a cell culture by a sphingolipid elicitor. Silencing of *OsRac1* by RNA interference resulted in a strong reduction of protein levels and kinase activation of OsMAPK6. Furthermore, coimmunoprecipitation experiments showed that OsMAPK6 is closely associated with the active form of OsRac1[31]. This is the first report about the association of a MAPK cascade and small G protein mediated immune response in plants, suggesting that OsRac1 possibly activate the MAPK signaling cascade, which in turn triggers the downstream effectors and leads to the immune response.

4.2.3 OsRac1 regulates the downstream signaling

events through forming the complex with molecular chaperones as well as scaffolding proteins In the signaling pathway, molecular chaperones are generally involved in the refolding and assembling of signal molecules. Among them, the Hsp90 family with a molecular weight of 90 kDa has been demonstrated play a crucial role in the R proteins mediated immune response against pathogens. These R proteins include Pto in tobacco, RPM1 and RPS2 in *Arabidopsis*[32]. Another two signaling component proteins, RAR1(required for Mla12 resistance), a Cys- and His-rich (CHORD) Zn^{2+} binding domains containing protein, and its interacting partner SGT1(for suppressor of the G2 allele of skp1), a homologue of the yeast ubiquitin ligase-associated protein, were also found as two essential factors required in plant disease resistance triggered by a number of R proteins[33,34]. In arabidopsis, it was reported that RAR1 and SGT1 function closely with Hsp90 to exert the chaperoning roles through formation of Hsp90-RAR1-SGT1 complex that are essential for disease resistance[32,35].

In rice, the similar role of RAR1 and SGT1 proteins played in rice innate immune responses has been described. Physical interaction of OsRAR1 and OsSGT1 *in vivo* and in yeast was detected and the overexpression of OsRAR1 and OsSGT1 in rice significantly increased basal resistance to bacterial blight and fungal blast[36]. Regulation of OsRac1 mediated immune response through the formation of complex by molecular chaperone, RAR1 and SGT1 was also studied. OsRAR1-RNAi rice lines showed impaired basal resistance to a compatible race of the blast fungus *M. grisea* and the virulent bacterial blight pathogen *Xoo*. The transgenic rice plants carrying both the CA-OsRac1 and OsRAR1-RNAi constructs, however, had the same level of resistance as untransformed control plants, indicating that RAR1 is required for OsRac1-mediated disease resistance. In addition, addition of Hsp90-specific inhibitor to the elicitor treated CA-OsRac1 cell cultures resulted in a substantial decrease in mRNA level of PBZ1 and Chitinase1, two defense response marker genes, suggesting that Hsp90 function is essential for OsRac1-mediated enhancement of PAMP-triggered immune responses. Further experiments with the method of coimmunoprecipitation showed that OsRac1 forms a complex with RAR1, Hsp90, and Hsp70 *in vivo*. The main function of Hsp90 is maybe to help the complex formation of OsRAR1 and OsRac1[37]. Very recently, we found that the Hsp90 cochaperone Hop/Sti1 was required for chitin-triggered immune responses and the Hop/Sti1 interacts with the OsRac1[38].

RACK1 (receptor for activated C kinase) is a multifunctional scaffolding protein known to be involved in the regulation of various signaling cascades including hormone signaling and development in plants[39]. Recently, by using the OsRac1 affinity column chromatography and mass spectrometry, a total of 21 bound proteins were identified. Among them, a scaffolding protein RACK1A was highlightly studied[40]. This study showed that RACK1A transcription was induced by a fungal elicitor and by abscisic acid, jasmonate and auxin. Transgenic rice overexpressing the RACK1A enhanced ROS production and increased resistance against rice blast infection. Interestingly, RACK1A was shown to interact with the N terminus of NADPH oxidase, RAR1, and SGT1[40]. As it is known, the scaffolding protein generally serves as a molecular glue for kinase anchoring and as an integrative point for diverse signal transduction pathways, in such way the specificity and efficiency of signal transduction was ensured[41,42]. According to this hypothesis and based on these results, we propose that rice RACK1A likely functions as a scaffold protein for the formation of an interactive complex including OsRac1,RAR1 and SGT1 and to maintain an effective conformation which is able to activate the downstream effectors and lead to the immune response.

4.2.4 The heterotrimeric G protein α subunit acts upstream the OsRac1 Heterotrimeric G proteins, a major group of signaling molecules for a variety of cellular activities and made up of alpha (α), beta (β) and gamma (γ) subunits, has also been demonstrated to be involved in the rice defense response. The rice Gα mRNA can be induced by fungal elicitor treatment and by infection with an avirulent race of funal blast pathogen.

In the rice dwarf1 (d1) mutants lacking a functional *Gα* gene, a reduced HR and delayed PR gene expression after blast infection in leaves and suppression of H_2O_2 production upon elicitor treatment in cultured cells were demonstrated, indicating the Gα plays an important role in rice defense system. Further experiment showed that transgenic d1 plants expressing the constitutively active OsRac1 can recover H_2O_2 production and PR gene expression, suggesting that the *Gα* gene acts upstream the OsRac1[25].

5 Conclusions and perspectives

Taken together, the main progress on the small GTPase mediated rice disease resistance is reflected by the findings of critical components involved in the OsRac1 mediated immune response. Although the OsRac1 signaling pathway is not very clear now, for instance the exact upstream activators and the downstream effectors of OsRac1 and the fine regulatory mechanisms employed are still unknown, the accumulated data could allow us to propose tentatively a working model for OsRac1 mediated signal transduction pathway in rice immune defense. The model could be described as follows: upon the infection by pathogen, the plant cell can recognize the ligands from pathogen via specific receptors on plasma membrane. Following the recognition, an intracellular regulator, maybe the heterotrimeric G-protein Gα subunit, is activated and which in turn transduces the signals to the small GTPase OsRac1. The active OsRac1 acts as a molecular switch to turn on the downstream effectors such as the enzymes involved in synthesis of defense related substances. This process was likely regulated by the formation of a transient protein conformation complex composed of scaffolding proteins and the molecular chaperones recruited by OsRac1. In this way, the OsRac1 pathway finally leads to onset of defense response against pathogen in rice (Fig.1).

Identification of these components involved in the OsRac1 signaling pathway sheds a light on the molecular mechanisms for understanding of small G protein mediated defense response against pathogen in rice, however, there is still considerable uncertainty as to the identity of the major components, which are likely to refine of even challenge the current model. One of the well conserved regulation mechanisms of small GTPase is that its activation is controlled by the specific GEF. GEFs activate GTPases by stimulating the exchange of GDP for GTP, thus allowing small GTPases to regulate downstream effectors. Recently, plant unique GEFs for small GTPase ROPs family were identified as a group of PRONE (plant-specific Rop nucleotide exchanger) domain containing proteins[43,44]. In rice eleven PRONE-type RacGEFs candidates have been found[45]. This, therefore, raises the question: among these GEFs which one is exclusively active towards OsRac1 and how does it finally regulate its GTPase activity and thus control the rice immune response? This is the key to fully understand the OsRac1 mediated rice defense mechanisms. Therefore the identification of OsRac1 specific GEF and the study of its regulation against rice immune system will be the challenging work in future.

In other plants increasing evidences also demonstrate the role of small GTPase played in the regulation of plant defense against pathogens. In tobacco, small G proteins, homologous to the neutrophil Rac2 protein Rac2, were found to be involved in elicitor-induced oxidative burst[46]. More recently, an *Arabidopsis* small GTPase RabG3b of Rab family was isolated as a salicylic acid-responsive protein and a modulator for cell death progression during pathogen response[47]. Interestingly, on the contrary, some RacB members of plant small GTPase play a negative role in regulation of disease resistance. For instance, the barley ROP protein HvRacB modulates susceptibility to the powdery mildew disease caused by *Blumeria graminis f.sp. hordei* (Bgh)[48,49] and its rice homolog OsRacB functions as a potential negative regulator for a basal disease resistance in rice[50].The contrary role played by different members within the same Rho family is an interesting question to answer, also indicating the complexity of small G proteins regulated plant disease resistance.

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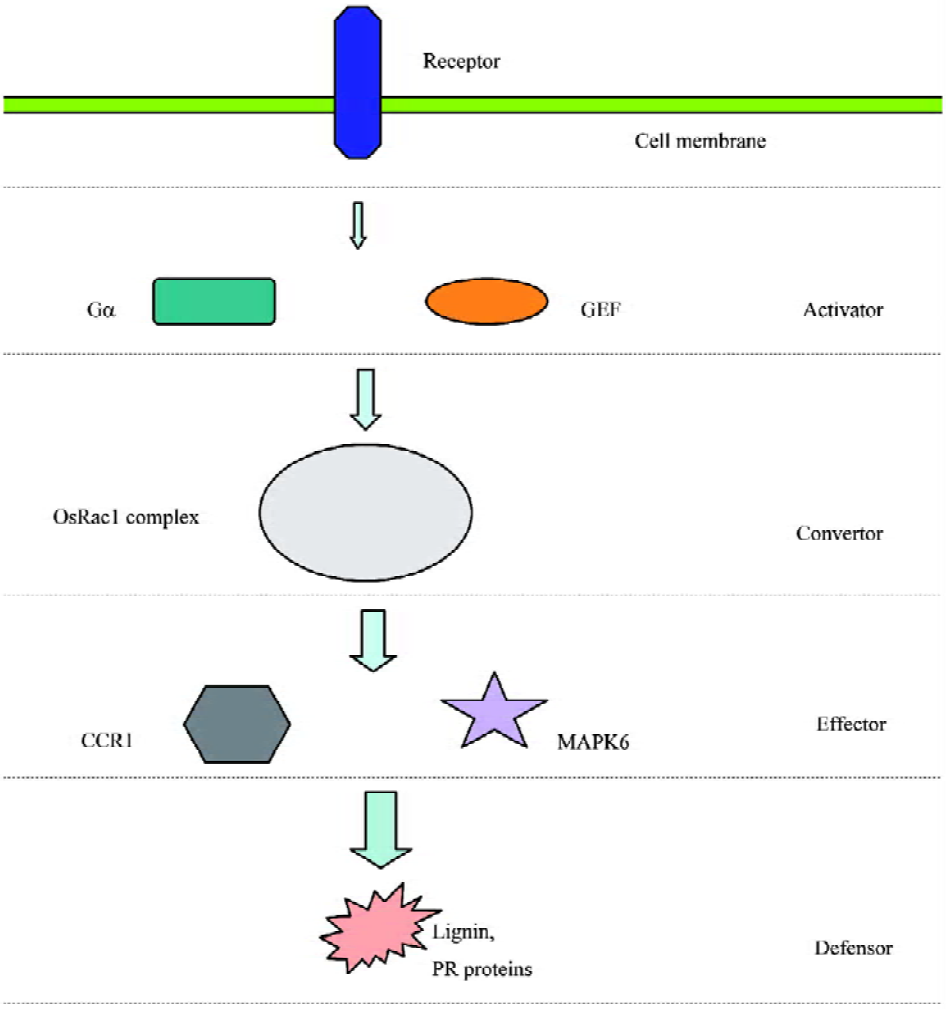

Fig. 1 Proposed signaling pathway for small GTPase OsRac1 mediated rice defense response

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小 G 蛋白在植物抗病性中的作用

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摘要 小 G 蛋白一类是低分子量 GTP 结合蛋白, 其分子量大约 20~30 kDa。小 G 蛋白作为重要的分子开关参与了细胞许多重要生理信号途径的调控。近几年在植物中的研究、尤其是对模式植物水稻抗病分子机制的研究发现, Rho家族的小G蛋白在植物抗病信号传导途径的调控中起了关键的作用。本文对植物特有的Rho家族小G蛋白在植物免疫反应中的最新研究进展进行了综述。

关键词 植物抗病性; 小 G 蛋白; 水稻

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