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RACK1, a Versatile Scaffold Protein in Plants?

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ABSTRACT

Receptor for Activated C Kinase 1 (RACK1) is a tryptophan-aspartic acid-domain (WD40) repeat protein. Compelling evidence supports the notion that RACK1 is a versatile scaffold protein which binds numerous signaling molecules from diverse signal transduction pathways and plays critical roles in multiple developmental processes in mammals. RACK1 orthologs are present in plants. In particular, the Arabidopsis genome contains genes that encode three RACK1 proteins, all of which are over 75% similar to mammalian RACK1 at the amino acid level. In addition, all functional domains of RACK1 protein including the number and position of WD40 repeats and the protein kinase C binding sites are largely conserved in plant RACK1 proteins. However, no signaling protein has been shown to physically interact with plant RACK1, therefore a scaffolding function of RACK1 protein has not been established in plant cells. Recently, the characterization of the first loss-of-function *RACK1* mutant, *rack1a*, in Arabidopsis has shed lights on a scaffolding function of RACK1 in plants. *rack1a* mutants display defects in multiple developmental processes and hormone responsiveness, consistent with a scaffolding role of RACK1. In this article, we provide a comprehensive review of RACK1 in plants, compare its domain structure with mammalian RACK1, and analyze which RACK1-interacting proteins may be conserved in plants. Future studies are expected to lead the discovery of a wide range of RACK1-interacting proteins and the determination of the molecular mechanism of the action of RACK1 in plant cells.

Keywords: 14-3-3 protein, Arabidopsis, eIF6, heterotrimeric Gβ subunit, PP2A, WD40 repeat

CONTENTS

INTRODUCTION

RACK1 was originally identified as a receptor for activated protein kinase C (PKC) (Ron *et al*. 1994). Now RACK1 has been recognized as a scaffold protein that has versatile roles in diverse signal transduction pathways. There are several excellent review articles that highlight the structure and function of RACK1 in mammals and yeasts (McCahill *et al*. 2002; Chen *et al*. 2004b; Sklan *et al*. 2006). Here we mainly focus on the study of RACK1 in plants, particularly in the model plant Arabidopsis. For details of RACK1 in other systems, readers are referred to the abovementioned review articles and the references therein. We begin with a brief review of the discovery and functional properties of RACK1 in mammalian cells.

DISCOVERY OF RACK1 AS AN INTRACELLULAR RECEPTOR

RACK1 gene was first cloned from a chicken liver cDNA library as a gene that is closely linked to the major histocompatibility complex loci (Guillemot *et al*. 1989). A few years later, Ron *et al*. (1994) isolated the *RACK1* gene from a rat brain cDNA expression library and proved that RACK1 protein fulfilled all the criteria for a receptor for activated PKC established by Mochly-Rosen *et al* (1991a, 1991b). Binding of RACK1 to the activated form of PKC anchors the latter to a sub-cellular location where its substrates are present (Ron *et al*. 1995). RACK1 was found to interact with both the "conventional (calcium-dependent)" and the "novel (calcium-independent)" PKC isoforms (Besson *et al*. 2002) with the conventional PKCβII isoform being the preferred binding partner (Stebbins and Mochly-Rosen 2001).

RACK1 IS A WD-REPEAT-CONTAINING PROTEIN

RACK1 is composed of seven tryptophan-aspartic acid-domain (WD40) repeats. Each WD-repeat spans approximately 40-60 amino acids. The WD domains start with a glycine-histidine (GH) dipeptide 11 to 24 residues from the N terminus and end with a tryptophan-aspartic acid (WD) dipeptide at the C terminus (Smith *et al*. 1999), though neither GH nor WD is absolutely conserved. A summary of

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Invited Review

some of the substitute amino acids at GH and WD positions of WD-repeat proteins is available at http://BMERC-www.bu.edu/wdrepeat (Smith *et al*. 1999).

The first WD-repeat-containing protein whose crystal structure was determined was the heterotrimeric G protein β subunit (Gβ) in which WD40 repeats form a seven-bladed β propeller structure (Wall *et al*. 1995; Sondek *et al*. 1996). Each WD domain contains the first three strands of one blade and the last strand in the next blade; the last WD domain comprised of the first three strands of the last blade and the last strand of the first blade to form a circular propeller structure. Other WD40-repeat-containing proteins whose crystal structures were resolved all form β propeller, indicating that the β propeller may be the predominant fold for this protein family (Robinson *et al*. 2001; Orlicky *et al*. 2003; Voegtli *et al*. 2003; Madrona and Wilson 2004).

RACK1 AS A SCAFFOLD PROTEIN

A scaffold protein is a protein whose main function is to bring other proteins together for them to interact. Besides its role as an intracellular receptor for activated PKC, RACK1 is capable of interacting with numerous other signaling molecules and modulating their cellular functions. As shown in **Table 1** and **Table 2**, most RACK1-interactors identified in mammals and fungi/yeasts are signaling proteins, such as receptors, kinases, phosphatases, transcription factors, and GTPases. These RACK1-interacting proteins include membrane-anchored proteins, cytosolic proteins, and nuclear proteins. Through the interaction with these partners, RACK1 plays regulatory roles in diverse developmental processes and physiological responses, such as cell cycle control, cell movement and growth, immune responsiveness, and neural responses (McCahill *et al*. 2002; Sklan *et al*. 2006).

Scaffolding property of RACK1 entitles it to integrate inputs from distinct signaling pathways. There are many examples that RACK1 functions as a scaffold protein to bring other proteins together to facilitate the interaction between them. For example, STAT1 (Signal Transducers and Activators of Transcription 1) is associated with one of its receptors, type I interferon (IFN), via RACK1 (Usacheva *et al*. 2001). Disruption of the interaction between RACK1 and IFNα receptor abolishes IFNα-induced tyrosine phosphorylation of STAT1. One way for RACK1 to scaffold two or more proteins together is through its ability to bind multiple proteins simultaneously. In consistent with this scenario, RACK1 has several independent protein binding sites (McCahill *et al*. 2002). On the other hand, two interacting proteins could have a same binding site on RACK1. RACK1 scaffolds their interaction by forming a homodimer to bring them together (Thornton *et al*. 2004).

DISCOVERY OF RACK1 IN PLANTS

RACK1 is highly conserved across several kingdoms of eukaryotic organisms including mammals, plants, fungi, fishes, and insects. Although not recognized as such, the first plant *RACK1* gene was cloned from tobacco BY-2 cells as an auxin (2,4-dichlorophenoxyacetic acid, 2,4-D) inducible gene, *arcA* (Ishida *et al*. 1993, 1996). Subsequently, a cDNA clone that encodes a protein that is highly similar to arcA was cloned from the greening leaves of rice (Iwasaki *et al*. 1995). Since then, RACK1 orthologs have been cloned in other plant species including alfalfa (McKhann *et al*. 1997), rape (Kwak *et al*. 1997), Arabidopsis (Vahlkamp and Palme 1997), and tomato (Kiyosue and Ryan 1999). RACK1 was also found in the green algae (Schloss 1990).

The completely-sequenced Arabidopsis genome contains genes that encode three RACK1 proteins, designated

Table 1 RACK1-interacting proteins in mammals and their homologs in Arabidopsis. NSH, no significant homologs, defined as sequence homology lower than 25% at the whole protein level.

	Reference	Arabidopsis homolog
MAMMALIAN PROTEINS		
Membrane anchored proteins/receptors		
RACK1	Thornton *et al*. 2004	Three Arabidopsis RACK1 genes share overall 65% identity to their human homolog
Gβ1γ1 heterodimer and Gα1β1γ1 heterotrimer	Dell *et al*. 2002	AGB1 (At4g34460) share 43% identity to bovine AGB1
ST7, a low-density lipoprotein receptor	Battle *et al*. 2003	NSH
Type I interferon receptor β long subunit (IFNαRβL)	Croze *et al*. 2000	NSH
Integrin	Liliental and Chang 1998	NSH
Insulin-like growth factor I (IGF-IR)	Hermanto *et al*. 2002	NSH
Angiotensin II receptor-associated protein	Wang *et al*. 2002	NSH
Beta chain of IL-5/IL-3/GM-CSF receptor	Geijsen *et al*. 1999	NSH
Inositol 1,4,5-trisphosphate receptors	Patterson *et al*. 2004	NSH
NR2B subunit of the NMDA receptor	Yaka *et al*. 2002	NSH
Androgen receptor (AR)	Rigas *et al*. 2003	NSH
Insulin receptor	Zhang *et al*. 2006	NSH
(GABA) gamma-aminobutyric acid type A receptor β1 subunit	Brandon *et al*. 1999	NSH
Na^+/H^+ exchange regulatory factor (NHERF1), a binding partner of CFTR	Liedtke *et al*. 2002	NSH
Kinases/Phosphatases		
Protein phophatase 2A (PP2A) heterodimer of catalytic subunit and regulatory A subunit	Kiely *et al*. 2006	Arabidopsis PP2A catalytic subunits and PP2A A subunits share about 80% and 56% identity to their human homologs
JNK1, JNK2, c-Jun N-terminal kinase, MAP kinases, specially activated by stress	Lopez-Bergami *et al*. 2005	Share above 34% identity to all the 20 MAP kinases in Arabidopsis
Activated Protein Kinase C (PKC)	Mochly-Rosen *et al*. 1991a	NSH
Src tyrosine kinase	Chang *et al*. 1998	NSH
Fyn tyrosine kinase	Yaka *et al*. 2002	NSH
Tyrosine kinase 2	Haro *et al*. 2004	NSH
Syndecan-2, a transmembrane heparan sulfate proteoglycan	Huang and Chuang 2006	NSH
PTPmu, receptor protein-tyrosine phosphatase	Mourton *et al*. 2001	NSH
Transcription factors		
p73α, tumor suppressor p53-related gene	Ozaki *et al*. 2003	NSH
STAT1, Signal Transducers and Activators of Transcription 1	Usacheva *et al*. 2001	NSH
STAT3	Zhang *et al*. 2006	NSH
pRB, retinoblastoma tumor suppressor	Ozaki *et al*. 2003	NSH
p63α, a homolog of tumor suppressor p53	Fomenkov *et al*. 2004	NSH

Table 2 RACK1-interacting proteins in mammals and fungi/yeasts and their homologs in Arabidopsis. NSH, no significant homologs, defined as sequence homology lower than 25% at the whole protein level.

	Reference	Arabidopsis homolog
MAMMALIAN PROTEINS (continued)		
Other proteins		
eIF6 translation initiation factor	Ceci *et al*. 2003	At3g55620 and At2g39820 share 72% and 58% identities to human eIF6
14-3-3β, regulatory factor that interacts with phosphorylated protein and affects their function	Chu *et al*. 2005	Thirteen expressed Arabidopsis 14-3-3 proteins share above 59% identity to mouse 14-3-3β
FAN, factor associated with neutral sphingomyelinase activation	Tcherkasowa *et al*. 2002	At2g45540 and At1g58230 share 35% and 33% identity to human FAN protein respectively
Oxysterol binding protein	Rodriguez *et al*. 1999	Twelve Arabidopsis oxysterol binding proteins (Skirpan *et al*. 2006) share 23% to 39% identity to their human homolog.
Phospholipase C-Gamma-1 (PLCγ1)	Disatnik *et al*. 1994	Arabidopsis PI-PLC isoforms are δ-type (Mueller-Roeber and Pical 2002)
Ras GTPase	Chu *et al*. 2005	No Ras GTPase in Arabidopsis (Vernoud *et al.* 2003)
Synaptic vesicle-specific p65 protein	Mochly-Rosen *et al*. 1992	NSH
Dynamin1	Rodriguez *et al*. 1999	NSH
β-Spectrin	Rodriguez *et al*. 1999	NSH
Ras-guanine nucleotide releasing factor (GRF)	Rodriguez *et al*. 1999	NSH
$p120^{GAP}$, a Ras GTPase-activating protein	Koehler *et al* 2001	NSH
p19, a Ras famlily protein	Guil *et al*. 2003	NSH
MURF1, muscle ring finger protein-1	Arya *et al*. 2004	NSH
cAMP-specific phosphodiesterase isoform PDE4D5	Yarwood *et al*. 1999	NSH
P0 (MPZ) myelin protein	Xu *et al*. 2001	NSH
Plectin	Osmanagic-Myers and Wiche 2004	NSH
Dopamine transporter (DAT)	Lee *et al*. 2004	NSH
acetylcholinesterase variant AChE-R	Perry *et al*. 2004	NSH
The prion-like protein doppel (Dpl)	Azzalin *et al*. 2006	NSH
hPER1	Hu *et al*. 2006	NSH
SMAD3	Okano *et al*. 2006	NSH
Ki-1/57, an intracellular hyaluronan-binding protein	Nery *et al*. 2004	NSH
FUNGUS/YEAST PROTEINS		
Gpa1, heterotrimeric G-protein α subunit	Palmer *et al*. 2006	Arabidopsis GPA1 share 35% identity to fungus Gpa1
Gpg1 and Gpg2	Palmer *et al*. 2006	NSH
(RanI) Pat I kinase	McLeod *et al*. 2000	NSH
Scp160p, a RNA binding protein	Baum *et al*. 2004	NSH
Msa2/Nrd1	Jeong *et al*. 2004	NSH
Pck2, protein kinase C homolog	Won *et al*. 2001	NSH
Pck1, protein kinase C homolog	Palmer *et al*. 2006	NSH
Smg1	Palmer *et al*. 2006	NSH

as RACK1A, RACK1B, and RACK1C (Chen *et al*. 2006). All three RACK1 proteins belong to the WD-repeat superfamily which contains 237 proteins in Arabidopsis (van Nocker and Ludwig 2003). Using a bovine Gβ whose crystal structure has been resolved (Lambright *et al*. 1996; Sondek *et al*. 1996) as a template, RACK1A was modeled as a 7-bladed β propeller structure (Chen *et al*. 2006) with each blade comprised of four anti-parallel β sheets as that found in bovine Gβ (Sondek *et al*. 1996).

The BLASTP search using Arabidopsis RACK1A protein (NCBI accession number: NP_173248) as a template revealed 15 RACK1 homologs in plants (**Fig. 1, Fig. 2**). Interestingly, besides Arabidopsis, other plant species also contain more than one copy of *RACK1* genes, in contrast to only one copy of *RACK1* gene in other organisms. For example, rice contains two RACK1 homologs, RWD1 and RWD2, which are approximately 80% similar to Arabidopsis RACK1 proteins at the amino acid level (**Fig. 3**).

All plant RACK1 proteins share over 65% identity and 80% similarity each other at the amino acid level when aligned with Blast 2 Sequences (Tatusova and Madden 1999). RACK1 homolog in *Brassica napus* shared the highest homology to Arabidopsis RACK1A (96% identity and 98% similarity) than to RACK1B and RACK1C (87% identity and 94% similarity). All plant RACK1 proteins are approximately 75% similar to mammalian RACK1 at the amino acid level (**Fig. 2**). The number and position of GH and WD core sequences as well as the PKC binding sites are largely conserved in plant RACK1 proteins (**Fig. 2**)

Despite of the highly conserved amino acid sequences of RACK1 proteins in different organisms, the region between the 6th and 7th conserved GH-WD core sequences displays high variation (**Fig. 2**). This region is the greatest variable region in all WD-repeat proteins and is predicted to be exposed to the surface of the 7-bladed propeller structure of the WD-repeat proteins, thus presumably determining their binding properties (Smith *et al*. 1999). In consis-

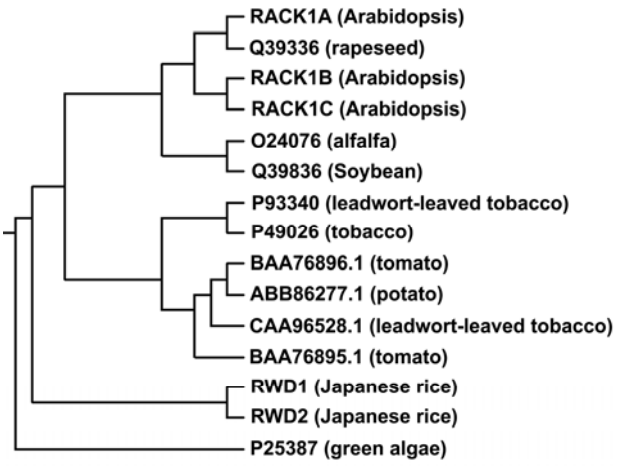

Fig. 1 The N-J phylogenetic tree of RACK1 orthologs in plants. Fifteen full-length proteins from 10 species were included. Each protein was labeled with an NCBI accession number or protein name followed by a common species name (in parentheses). The N-J phylogenetic tree was generated by GenomeNet CLUSTALW Server (http://clustalw.genome.jp/).

Fig. 2 Protein sequence alignment between plant RACK1 orthologs and mammalian RACK1. The NCBI accession number or protein name is indicated at the beginning of each sequence. Proteins analyzed include RACK1A (Arabidopsis, NCBI accession number NP_173248), RACK1B (Arabidopsis, NCBI accession number NP_175296), RACK1C (Arabidopsis, NCBI accession number NP_188441), Q39336 (rapeseed), O24076 (alfalfa), Q39836 (soybean), BAA76896.1 (tomato), BAA76895.1 (tomato), CAA96528.1 (leadwort-leaved tobacco), P93340 (leadwort-leaved tobacco), ABB86277.1 (potato), ABB02625.1 (potato), P49026 (tobacco), RWD1 (Japanese rice), RWD2 (Japanese rice), P25387 (green algae), and human RACK1 (NCBI accession number P25388). The positions for conserved GH and WD dipeptides are indicated by "##" and "**" respectively on the top of the sequences. The conserved PKC binding domains are indicated by blocks on the top of the sequences. Within each sequence, identical amino acids among RACK1 proteins are shown as dots, and similar amino acids are shaded by grey color. Gaps are shown as dashed lines. All sequence alignments in this article were generated by the ClustalW multiple alignment of BioEdit Sequence Alignment Editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The positions for WD repeat domains were obtained from the SMART database (http://smart.embl-heidelberg.de).

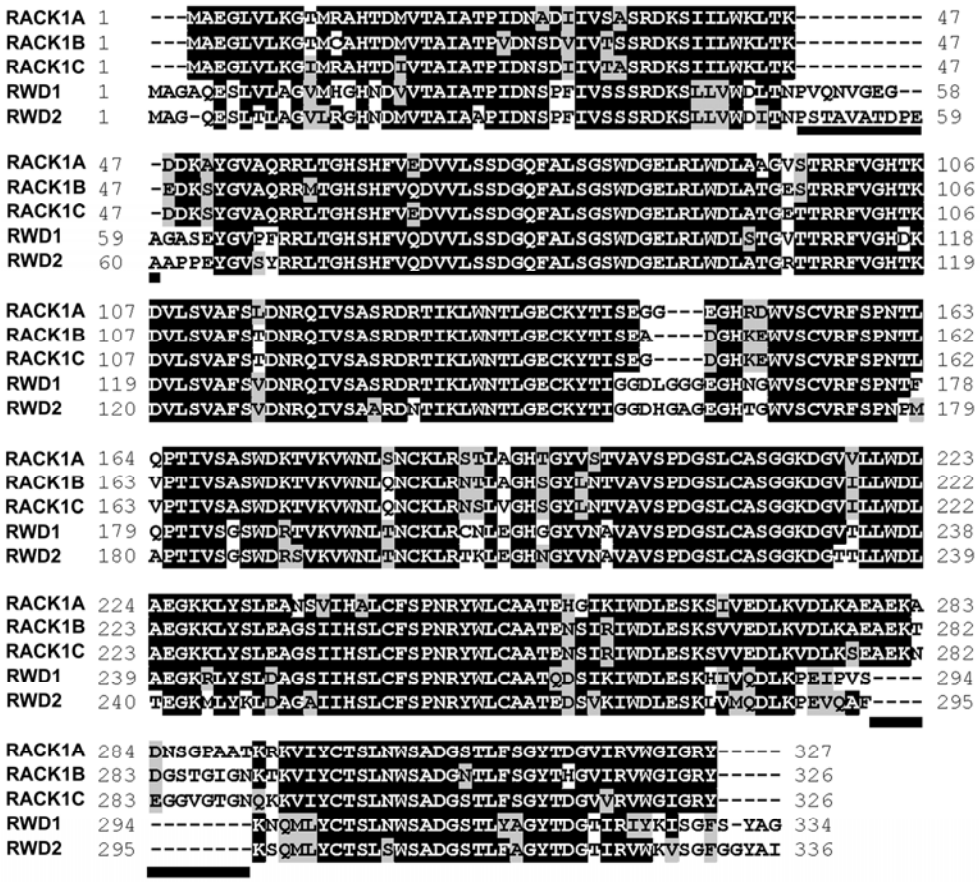

Fig. 3 Protein sequence alignment between RACK1 proteins in Arabidopsis and rice. The NCBI accession numbers for rice RACK1 proteins, RWD1 and RWD2, are NP_001043910.1 and NP_001056254.1. The highest variation in sequence between Arabidopsis and rice RACK1 proteins are underlined.

tent with this scenario, the majority of RACK1-interacting partners bind the 6th and 7th WD repeats of RACK1 protein (McCahill *et al*. 2002). Interestingly, two RACK1 proteins, RWD1 and RWD2, in rice, the only monocot plant in our list, have distinctive sequences between the 2nd and 3rd conserved GH-WD core sequences while have shorter sequence between the 6th and 7th GH-WD core sequences when compared with RACK1 proteins in Arabidopsis and other dicot plants (**Figs. 2, 3**). These distinctive run of sequences suggest that there may exist some distinctive binding partners for rice and Arabidopsis RACK1 proteins.

FUNCTIONAL CHARACTERIZATION OF RACK1 IN PLANTS

The biological function of RACK1 was proposed to be established prior to the separation of plant and animal kingdoms approximately 600 million to 1 billion years ago (Neer *et al*. 1994). In mammals and human, *RACK1* was expressed ubiquitously in different tissues and organs including brain, liver and spleen, consistent with its scaffolding function (Chou *et al*. 1999).

Because *RACK1* was originally identified as an auxin-inducible gene in tobacco BY-2 suspension cells in a differential screen for genes involved in auxin-mediated cell division (Ishida *et al*. 1993), RACK1 was proposed to have a role in auxin-mediated cell division. The induction of *RACK1* (*ArcA*) transcription in the BY-2 cells was specific to auxin, but not to other plant hormones, such as abscisic acid, gibberellic acid, ethylene, and cytokinin (benzylaminopurine, BAP). Interestingly, the transcription of *Msgb1*, a RACK1 ortholog in alfalfa, was induced by BAP, but not by 2,4-D in roots (McKhann *et al*. 1997).

Kwak *et al*. (1997) observed that when the RACK1 ortholog in *Brassica napus* L. was injected into *Xenopus laevis* oocytes, the insulin-induced maturation of oocytes, a PKC-mediated pathway, was inhibited, mimicking the effect of rat RACK1 (Smith and Mochly-Rosen 1992). This result implies that plant RACK1 may have a conserved function by acting as an intracellular receptor for activated PKC, though PKC ortholog in plants has yet to be identified.

Perennes *et al*. (1999) found that *RACK1* was induced by Ultra Violet (UV) treatment in tobacco BY-2 cells and this induction could be blocked by salicylic acid (SA) treatment. Because UV irradiation and SA acted as agonists to arrest BY-2 cells at cell cycle entry, and *RACK1* transcript was induced at cell cycle entry, it was hypothesized that RACK1 is involved in UV and SA mediated-cell cycle arrest (Perennes *et al*. 1999).

Komatsu *et al*. (2005) used a proteomic approach to analyze protein expression profiles in the embryos of rice *d1* mutant, a loss of function mutant of the heterotrimeric G protein α subunit (Gα). RACK1 was found to be one of the seven proteins whose expression is down-regulated in *d1* mutant. RACK1 protein was induced by abscisic acid in imbibed seeds of wild-type, but not in the *d1* mutant. Based on these results, the author proposed that the expression of RACK1 is regulated by Gα and that RACK1 may play important roles in rice embryogenesis and germination (Komatsu *et al*. 2005).

Chen *et al*. (2006) provided direct genetic evidence of the function of RACK1 in plants by characterizing the loss-

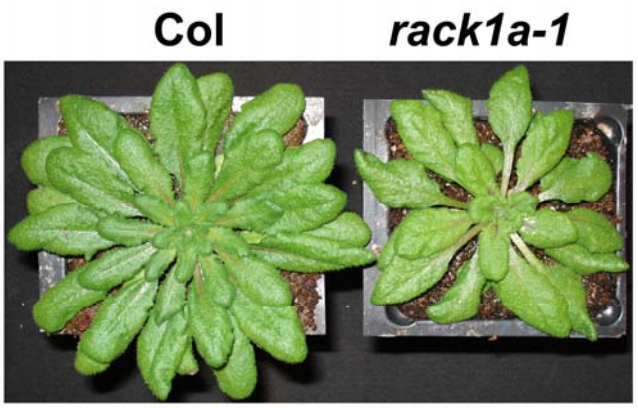

Fig. 4 Loss-of-function *RACK1* mutant in Arabidopsis. Wild-type Columbia-0 (Col, left) and *rack1a-1* mutant (right) were photographed 52 days after being grown under short-day conditions (8/16 h photoperiod).

of-function mutants of *RACK1* in Arabidopsis. Like mammalian *RACK1*, Arabidopsis *RACK1* genes express ubiquitously. Knocking out one of the three Arabidopsis *RACK1* genes, *RACK1A*, conferred defects in multiple developmental processes and resulted in pleiotropic phenotype (**Fig. 4**). The *rack1a* mutants have shorter hypocotyls in etiolated seedlings and epinastic cotyledons in light-grown seedling. When grown under short day conditions, *rack1a* mutants were late flowering and the rate of rosette leaf production was reduced by approximately 40%. Furthermore, *rack1a* mutants displayed altered sensitivities to several plant hormones, including hyposensitivity to gibberellic acid and brassinolide in seed germination, hyposensitivity to auxin in adventitious and lateral root formation, and hypersensitivity to abscisic acid in seed germination and early seedling development (Chen *et al*. 2006). The pleiotropic phenotype of *rack1a* mutants is consistent with a scaffolding function of RACK1.

RACK1 INTERACTING PARTNERS IN PLANTS

Although RACK1 has been shown to interact with numerous proteins with diverse functions in mammals, little is known about RACK1-interactors in plants. Recently Chang *et al*. (2005) used a proteomic approach to demonstrate that RACK1 proteins are associated with the 40S subunit of cytosolic ribosome in Arabidopsis. Giavalisco *et al*. (2005) reported that RACK1 proteins co-migrate with the 80S ribosome in Arabidopsis. These two independent studies provided the first biochemical evidence that at least some parts of RACK1's function are conserved in plants, because RACK1 proteins are associated with ribosomes in both mammals and yeasts (Link *et al*. 1999; Ceci *et al*. 2003; Shor *et al*. 2003; Nilsson *et al*. 2004; Sengupta *et al*. 2004).

Fig. 5 Protein sequence alignment between eIF6 proteins in Arabidopsis and human. The NCBI accession numbers for Arabidopsis eIF6 proteins, AteIF6-1 and AteIF6-2, are NP_181512.1 (At2g39820) and AAP75806.1 (At3g55620). The NCBI accession number for human eIF6, heIF6, is AAK39426.

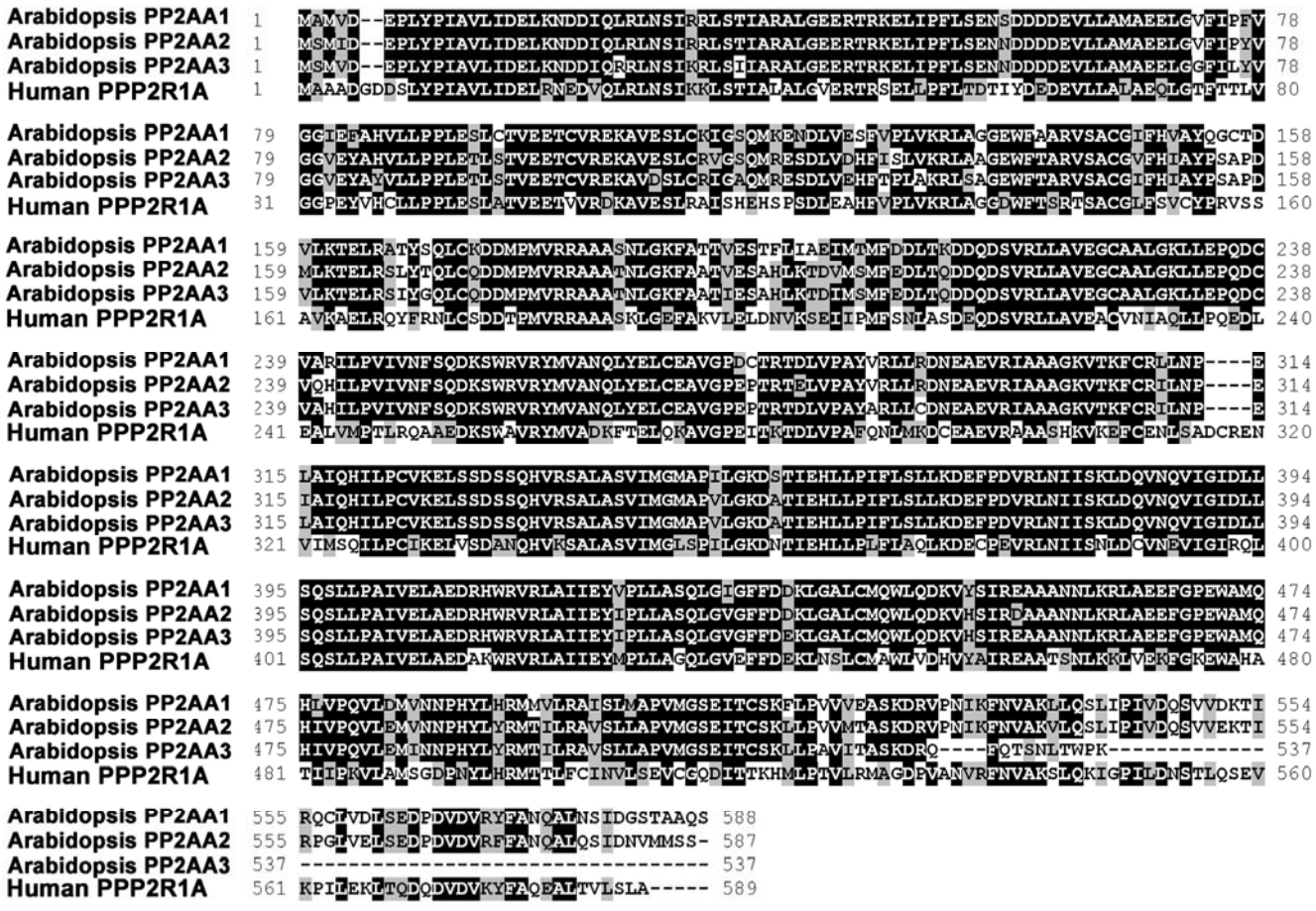

Fig. 6 Protein sequence alignment between protein phosphatase 2A-A subunits in Arabidopsis and human. The NCBI accession numbers for Arabidopsis PP2A A subunits, PP2AA1/RCN1, PP2AA2, and PP2AA3, are Q38845 (At1g25490), AAP37715.1 (At3g25800), and NP_001031035.1 (At1g13320). The NCBI accession number for human PP2A A subunit α isoform, PPP2R1A, is P30153.

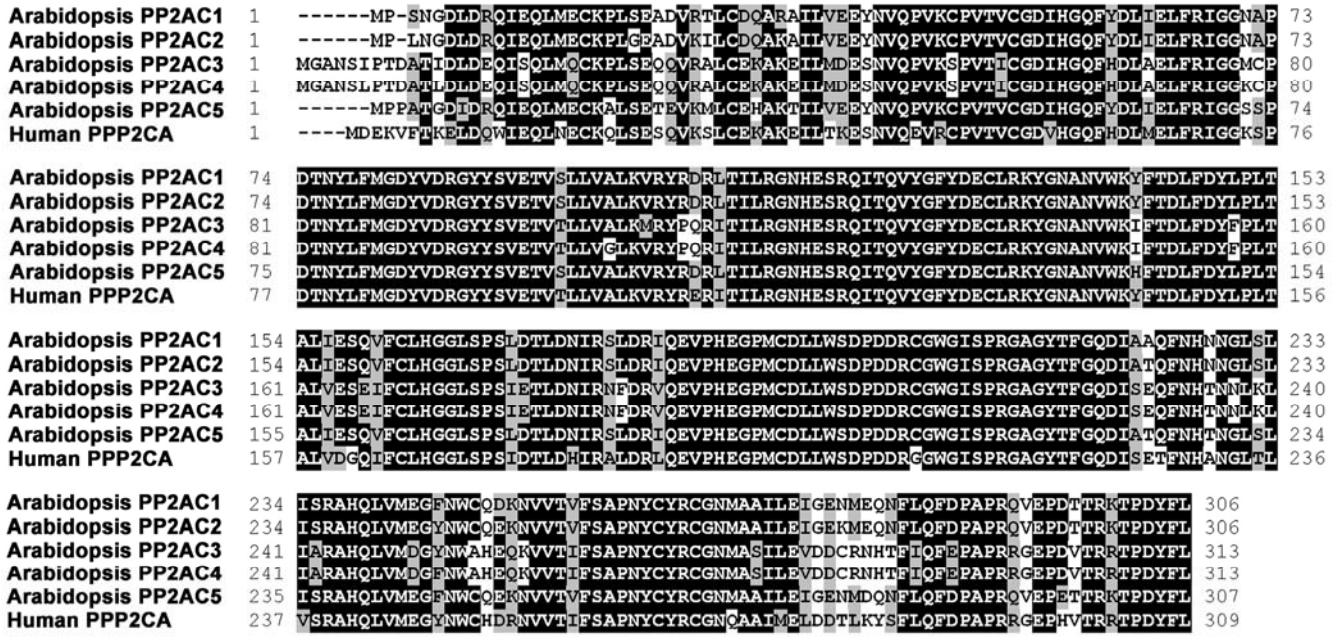

Fig. 7 Protein sequence alignment between protein phosphatase 2A-C subunits in Arabidopsis and human. The NCBI accession numbers for Arabidopsis PP2A C subunits, PP2AC1 to PP2AC5, are Q07098 (At1g10430), Q07099 (At1g59830), Q07100 (At2g42500), P48578 (At3g58500), and ABF85773.1 (At1g69960). The NCBI accession number for human PP2A C subunit α isoform, PPP2CA, is NP_002706.1.

Fig. 8 Protein sequence alignment between Arabidopsis Gβ, AGB1 (NCBI accession number: NP_195172.1) and bovine Gβ, GNB1 (NCBI accession number: P62871). Boxed are the RACK1-interacting domains mapped by Dell *et al*. (2002).

Interestingly, among approximately 60 RACK1-interacting proteins identified thus far in mammals and yeasts, only a few of them have significant homologs in plants (**Tables 1, 2**). On the basis of BLAST search analysis of the NCBI Arabidopsis protein database using each mammalian or fungus/yeast RACK1-interacting protein as a template, we found that mammalian RACK1-interacting proteins eIF6, protein phosphatase 2A (PP2A), 14-3-3β, and Gβ have highest homologies in Arabidopsis (**Figs. 5-8**). In mammalian cells, eIF6 translation initiation factor binds free 60S ribosome subunit and keeps the 40S and 60S subunits from assembling into a functional 80S ribosome. RACK1 functions as a physical linker to bring activated PKC and its substrate eIF6 together and lead to the phosphorylation of eIF6 by PKC; the phosphorylated eIF6 could eventually dissociate from the 60S subunit and allow joining of the two ribosomal subunits (Ceci *et al*. 2003). The RACK1 homolog in yeast, Asc1p/Cpc, is also associated with the ribosome (Shor *et al*. 2003) and coprecipitated with eIF6 complex (Volta *et al*. 2005). Mutation in Asc1p led to an impaired 80S formation and a reduced efficiency of translation (Chantrel *et al*. 1998). There are two eIF6 homologs (At3g55620 and At2g39820) in Arabidopsis. Mammalian eIF6 has 72% identity and 84% similarity with At3g55620, and 58% identity and 77% similarity with At2g39820 at the amino acid level (**Fig. 5**). Because RACK1 proteins have been found to be associated with the small ribosomal subunit in Arabidopsis (Chang *et al*. 2005) and in algae (Manuell *et al*. 2005), a role of RACK1 in the regulation of translation may be evolutionarily conserved accross kingdoms. Further studies are required to examine a direct interaction between RACK1 and eIF6 proteins in plant cells.

The second promising candidate interactor of RACK1 in plants is the PP2A, an intracellular serine/threonine protein phosphatase. In mammalian cells, PP2A holoenzymes exist as either a heterodimer consisting of a 36-KDa catalytic subunit (C subunit) and a 65-kDa regulatory A subunit, or as a heterotrimer consisting of this heterodimer and one of the regulatory B subunits (Xu *et al*. 2006). While the regulatory A subunit is required for scaffolding the PP2A holoenzyme heterotrimer, the regulatory B subunit regulates the subcellular location and specificity of the enzyme (Sheng 2003). RACK1 was found to interact with the heterodimer of PP2A comprising of the regulatory A subunit and the catalytic C subunit (Kiely *et al*. 2006). The Arabidopsis genome contains three PP2A subunits, 17 B subunit, and five C subunits, which can be theoretically assembled into 255 heterotrimeric PP2A isoforms (Zhou *et al*. 2004). These three Arabidopsis PP2A A subunit isoforms, PP2AA1, PP2AA2, and PP2AA3, exhibit approximately 56% identity with the human homolog at the amino acid level (**Fig. 6**). The five Arabidopsis PP2A C subunit isoforms, PP2AC1 to PP2AC5, exhibit about 80% identity with the human C subunit α isoform (**Fig. 7**). Genetic study of Arabidopsis PP2A A subunit mutant, *rcn1* (*root curl in naphthylphthalamic acid 1*), and B subunit mutant, *ton2* (*tonneau 2*), revealed that PP2A and its regulatory subunits are crucial for plant development and hormonal signaling (DeLong 2006). It would be interesting to determine if

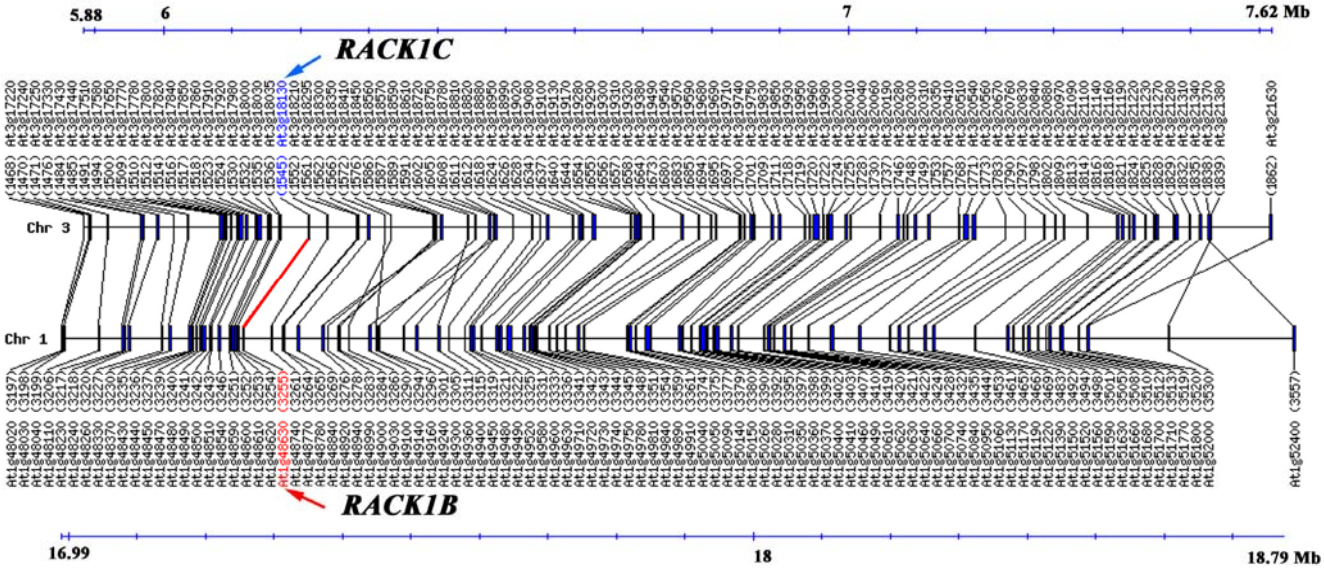

Fig. 9 Illustration of genomic duplication of *RACK1B* and *RACK1C* genes. *RACK1B* (At1g48630) and *RACK1C* (At3g18130) sit in the genomic duplication block # 0103319703610. The graph was generated from http://wolfe.gen.tcd.ie/athal/index.html (Blanc *et al*. 2003).

RACK1 can interact with PP2A A and C subunits in plants.

The 14-3-3β protein was identified as a RACK1 interactor in mouse cells (Chu *et al*. 2005). 14-3-3 proteins are highly conserved in eukaryotes. Their general function is to specifically bind to phosphorylated proteins and change their activity, stability or localization (Ferl 2004). The discovery of the function of 14-3-3 proteins reinforces the long-time held concept that phosphorylation/dephosphorylation of the target protein is sufficient to change its activity. There are 15 14-3-3 genes in Arabidopsis genome and at least 13 of them are expressed. Arabidopsis 14-3-3 proteins share over 50% identity at the amino acid level each other, and can be divided into two major evolutionary groups, ε group and non-ε group, based on the phylogenetic analysis (Sehnke *et al*. 2002). Results from tissue and subcellular localization, genetic study, and biochemical binding assays suggested the presence of both overlapping and distinct functions among 14-3-3 proteins in plants (Sehnke *et al*. 2002). Several important roles of plant 14-3-3 proteins have been revealed. For example, binding of phosphorylated nitrate reductase (NR) to 14-3-3 proteins and divalent cations inactivates the enzyme activity of NR (Huber *et al*. 1996). 14-3-3s and Mg^{2+} can also bind the H^{+}-ATPase and stimulate its pump activity (Malerba and Bianchetti 2001). The mouse 14-3-3β which interacts with RACK1 is more closely related to the non-ε group 14-3-3 proteins (over 59% identity at the amino acid level). However, the interaction between 14-3-3 proteins and RACK1 has yet to be determined in plant cells.

Another promising candidate interactor of RACK1 in plants is the Gβ subunit. Heterotrimeric G proteins are evolutionarily conserved in all eukaryotes (Temple and Jones 2007). Arabidopsis Gβ, AGB1, shares 43% identity and 61% similarity with bovine Gβ protein at the amino acid level (**Fig. 8**). In mammalian cells, binding of RACK1 to Gβγ results in a specific inhibition of Gβγ-mediated activation of PLCβ2 and adenylyl cyclase II whereas has no effect on other functions of Gβγ (Dell *et al*. 2002; Chen *et al*. 2004a). Dell *et al*. (2002) mapped the Gβ-interacting region on RACK1 to the 207 amino acids at the N-terminus of RACK1. The amino acid sequences that is most divergent between mammalian RACK1 and Arabidopsis RACK1 proteins (the amino acid 280-300 region, **Fig. 2**) is not required for its interaction with Gβ (Dell *et al*. 2002). Chen *et al*. (2005) mapped the RACK1-interacting regions on Gβ and identified five amino acid segments that are important for the interaction. These amino acid segments of Gβ are approximately 38% to 90% identical to those in Arabidopsis Gβ (**Fig. 8**). Plant heterotrimeric G proteins play important roles in multiple developmental processes, hormonal responsiveness, and stress responses (Perfus-Barbeoch *et al*. 2004). As mentioned above, RACK1 protein was down-regulated in the embryos of rice Gα mutant (Komatsu *et al*. 2005), raising the possibility that the interaction between RACK1 and the heterotrimeric G proteins might be conserved in plants. Such an interaction deserves further investigation.

Palmer *et al*. (2006) reported that a RACK1 homolog in the fungus *Cryptococcus neoformans*, Gib2, can function as an atypical heterotrimeric Gβ subunit and interact with one of the three Gα homologs, Gpa1, and two Gγ homologs, Gpg1 and Gpg2 (Palmer *et al*. 2006). However, in mammalian cells, RACK1 only interacts with Gβ1γ1 heterodimer and Gα1β1γ1 heterotrimer, but not with Gα subunit alone (Dell *et al*. 2002). Similarly, RACK1A does not interact with the sole Gα in Arabidopsis in a yeast split-ubiquitin assay (Chen *et al*. 2006).

CONCLUDING REMARKS

The study of RACK1 in plants is still at its fetal stage. Despite a long list of RACK1-interacting proteins in mammals, most of these interactors do not have significant homologs in plants. Therefore it remains mysterious if RACK1 could also function as a scaffold protein in plant cells. The identification and characterization of the whole spectrum of RACK1's physical interacting partners in plants will be of great importance to unravel the molecular mechanism of the action of RACK1 in plants. If the scaffolding function of RACK1 is indeed conserved in plants, we would expect to identify a wide range of RACK1-interacting proteins. Future studies should also focus on the determination of the physiological pathways in which RACK1 plays a regulatory role and the signals which regulate others via the scaffolding properties of RACK1. In addition, because most plant species have more than one *RACK1* gene whereas there is only one in other organisms, it is essential to clarify a potential functional redundancy among these genes. At least in Arabidopsis, two of three *RACK1* genes, *RACK1B* and *RACK1C*, were a consequence of chromosomal segment duplication (**Fig. 9**). *RACK1B* and *RACK1C* sit in the duplicated chromosomal segments corresponding to the most recent polyploidy in Arabidopsis genome which occurs sometime between 24 and 40 million years ago before the split of the Arabidopsis and *Brassica* lineages (Blanc *et al*. 2003). Because the formation of homodimer is one of the mechanisms through which RACK1 scaffolds other proteins' interaction in mammalian cells, the unique feature of multiple

RACK1 proteins in plant cells offers extra opportunities for forming heterodimeric complex among RACK1 proteins. Three Arabidopsis RACK1 proteins have variations in the major protein-interacting regions (**Figs. 2, 3**), implying that each RACK1 protein may have diverse binding partners. These diverse proteins could bind different RACK1 proteins and interact with each other through the platforms of homodimeric and heterodimeric complexes formed by RACK1 proteins. Such homodimeric and heterodimeric complex may help achieve a maximal scaffolding function of RACK1 in plant cells.

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