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Plant Rho-type (Rop) GTPase-dependent activation of receptor-like cytoplasmic kinases in vitro

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ABSTRACT

Plants have evolved distinct mechanisms to link Rho-type (Rop) GTPases to downstream signaling pathways as compared to other eukaryotes. Here, experimental data are provided that members of the Medicago, as well as Arabidopsis, receptor-like cytoplasmic kinase family (RLCK Class VI) were strongly and specifically activated by GTP-bound Rop GTPases in vitro. Deletion analysis indicated that the residues implicated in the interaction might be distributed on various parts of the kinases. Using a chimaeric Rop GTPase protein, the importance of the Rho-insert region in kinase activation could also be verified. These data strengthen the possibility that RLCKs may serve as Rop GTPase effectors *in planta*.
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1. Introduction

Rho-type GTPases belong to the Ras superfamily of small GTP-binding proteins which serve as two-state molecular switches depending on their GDP- or GTP-bound conformation [1,2]. Rho GTPases are implicated in diverse cellular processes through the regulation of cytoskeletal organization and dynamics, NADPH oxidase activity and gene expression [3,4]. Therefore it is not surprising that the GTP-binding and hydrolyzing activity of these multifunctional proteins is tightly regulated by a dedicated group of protein factors [5]. In addition to upstream regulators, the effector proteins downstream of Rho GTPases are also numerous and further increase the specificity of Rho GTPase signaling [6,7].

Rho-type small GTPases are ancient proteins present in most eukaryotes with considerable structural conservation. However, the early split of viridiplantae form the animal-fungal-amobeozoa lineage led to the separated evolution of plant Rop (as Rho-of-plants) GTPases [8]. This separation resulted in the accumulation of unique features as the primary structure, regulation and signaling properties of Rop GTPases are considered [8,9].

Among others, no Cdc42/Rac-interactive-binding (CRIB) motif-containing kinases (p21-activated kinases or PAKs), characteristic for other eukaryotes [6,10], could be identified in plants. PAKs play key roles in fundamental and general cellular processes, such as cytoskeletal rearrangements and the stimulation of mitogen-activated protein kinase (MAPK) cascades, in animal and yeast cells [10–12]. Therefore it is striking that similar pathways have not been identified in plants up to now. Moreover, plants do not have cognate Ras GTPases although these proteins are also key elements of MAPK-mediated mitogen signaling in other eukaryotes [13]. It is generally believed therefore that Rop GTPases, as the only signaling-type small GTPases in plants with combined Rho and Ras functions, should be linked to kinase cascades in a presently unknown way [14,15].

Here experimental evidences are presented that kinases belonging to the RLCK Class VI family of *Medicago truncatula* and *Arabidopsis thaliana* can be specifically activated by GTP-bound Rop GTPases in vitro further supporting the view that plant Rho GTPases may directly regulate downstream kinase signaling.

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2. Materials and methods

2.1. Site-directed mutagenesis and vector construction

Constitutive active (CA, $G_{15}V$) and dominant negative (DN, $T_{20}N$) mutant forms of the MsRop6 GTPase have been constructed by polymerase chain reaction (PCR) using oligonucleotid primers carrying the appropriate nucleotide changes. The cloning strategy was the same in all cases: two separate PCR reactions were carried out using one of the two overlapping primers carrying the appropriate mutation (Mut-fw and Mut-rev primers) together with the appropriate primer planned either to the start (Full-fw) or to the end (Full-rev) of the cDNA. The PCR products representing the overlapping 5′ and 3′ parts of the mutated cDNA were purified, mixed and the whole cDNA was amplified by the Full-fw and Full-rev primers. To introduce the C-terminal $C_{194}G$ mutation, a modified Full-revL primer was used. The Full-fw and Full-rev primers were extended by appropriate restriction sites to facilitate further cloning.

In order to change the MsRop6 Rho-insert region (amino acids 123–140) for the appropriate region of the human Ras protein (mutation ΔI^{Ras}), the PCR products (Mut-fw ΔIRas (*Pst*I) – Full-rev(*Sal*I) and Mut-rev ΔIRas(*Pst*I) – Full-fw(*Nde*I)) were cloned in one step into the NdeI/XhoI-digested pET26b vector (Novagen). Plasmids carrying the combined insert were digested by PstI, the overhanging nucleotides were removed by the large subunit of Klenow polimerase and the vector was recirculized by ligation.

All constructs have been verified by sequencing. For the primer sequences see Supplementary material 1.

2.2. Yeast two-hybrid screening

For the yeast two-hybrid screening experiment the vector construct pBD-GAL4 2.1/MsRop6CA,L was used as a bait and the *M. truncatula* root-nodule cDNA library published by Györgyey et al. [16] cloned into the pAD-GAL4 2.1 vector was used as prey. The vectors were purchased from Stratagen. The *Saccharomyces cerevisiae* yeast strain PJ69-4a [17] was used as host. Yeast transformation was done as described in [18]. The transformants were grown on appropriate drop-out media to monitor transformation efficiency as well as the activation of the HIS3 (in the presence of 1 mM 3-aminotriazol) and/or the ADE2 reporter genes.

Pairwise interaction analysis was carried out on the same way using individual cDNAs cloned into pBD-GAL4 2.1 and pAD-GAL4 2.1 vectors, respectively.

Production of the appropriate fusion proteins in the yeast cells has been verified by protein purification and Western blotting according to the protocols in the Yeast Protocols Handbook (Clonetech; www.clontech.com/images/pt/PT3024-1.pdf; February 2008). Antibodies against the GAL4 activation domain as well as for plant Rop GTPases have been purchased from Sigma.

2.3. Sequence analysis

Comparison and phylogenetic analysis of the protein sequences have been made as described elsewhere in details [19].

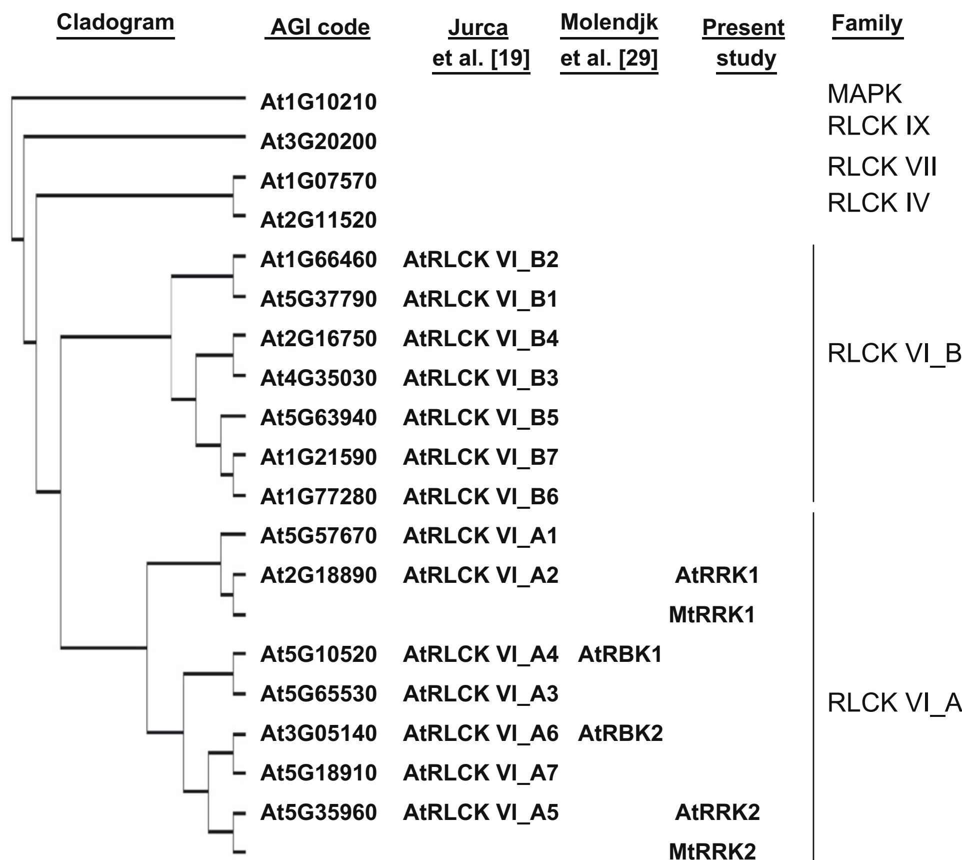

Fig. 1. Comparison and nomenclature of Arabidopsis and Medicago RLCK Class VI proteins. Similarity cladogram of the amino acid sequences of the 14 *Arabidospsis thaliana* and 2 *Medicago truncatula* RLCK VI and three other randomly selected Arabidosis RLCK kinases. The Arabidopsis mitogen-activated protein kinase (MAPK1; At1G10210) was used as an out-group. AGI codes and the nomenclature of the RLCK VI kinases used in the literature are also indicated. The RLCK VI groups (A and B) were defined and designated according to Jurca et al. [19].

2.4. Protein production and purification

Rop GTPases have been cloned into the vectors pMAL2c (N-terminal maltose-binding protein, MBP; New England Biolabs), pTRC-HIS C (N-terminal 6 × HIS tag; Invitrogen) and pET26b (C-terminal 6 × HIS, Novagen), while the RLCK-coding cDNAs have been inserted into pET28a (N-terminal 6 × HIS, Novagen).

Protein production was achieved in the *Escherichia coli* strain Rosetta™ [BL21 (DE3)/(pLysS)] (Novagen) following three or half an hour induction by 0.5 mM isopropyl-l-thio-/3-D-galactopyranoside (IPTG) in the case of GTPases or kinases, respectively. Purification of MBP-tagged proteins has been carried out as recommended by New England Biolabs. HIS-tagged protein purification using His-Select Sepharose (Sigma) has been modified by using the following buffers: for binding: 20 mM Tris–HCl pH 8.0, 200 mM NaCl, 5 mM imidazole; for washing: 20 mM Tris–HCl pH 8.0, 200 mM NaCl, 20 mM imidazole, 0.05 mM Tween®20; for elution: 20 mM Tris–HCl pH 8.0, 200 mM NaCl, 120 mM imidazole. Eluted proteins have been concentrated by CentriCon filters (Millipore) and stored in 50% glycerol at −20 °C.

2.5. Western blotting and in vitro protein interaction

Western blotting was carried out using standard procedures as described elsewhere in details [20]. Peroxidase-conjugated anti-poly histidine and anti-rabbit IgG antibodies have been purchased from Sigma, while the anti-maltose-binding protein serum from New England Biolabs. Immunoreactive bands were visualized by chemiluminescence using Lumi-Light Western Blotting Substrate (Roche) and X-ray films (Kodak).

In vitro protein pull down assays have been carried out exactly as described by Fülöp et al. [21].

2.6. Kinase activity measurements

The following reaction mix was set up: 2 pmol purified kinase, 5 pmol purified GTPase, 20 mM Tris–HCl pH 7.6, 5 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, 10 μM ATP, 0.2 MBq [γ-32P] ATP, and 0.25 μg/μl myelin basic protein. The reactions were stopped by 5 μl 5 × SDS loading buffer after 30 min at room temperature. Proteins were separated on SDS–polyacrylamide gels and the dried gels were analysed by a Phosphorimager 445 SI station (Molecular Dynamics).

2.7. Phosphorylation site determination

Phosphorylated residues of the in vitro phosphorylated, gel-purified, HIS-tagged MtRRK1 kinase and MsRop6 GTPase have been determined by MALDI-TOF and LC–MS/MS as described in details by Hlavanda et al. [22].

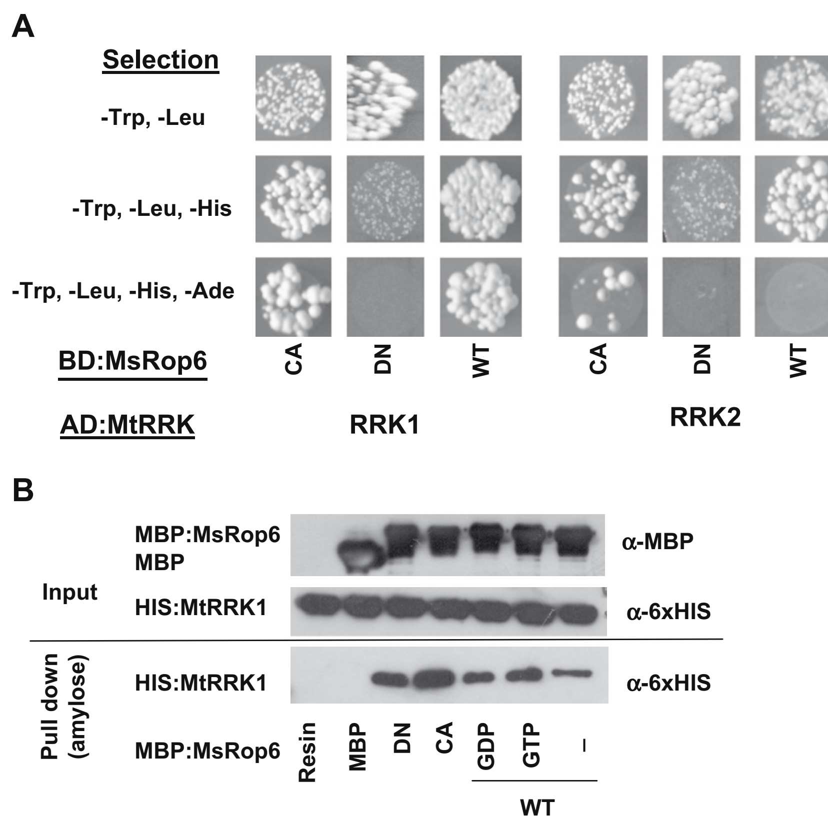

Fig. 2. Interaction of MtRRK kinases with various forms of the MsRop6 GTPase in yeast and in vitro. (A) Pairwise yeast two-hybrid interaction of the MtRRK1 and MtRRK2 kinases (as preys), respectively, with the wild type (WT), constitutive active (CA) or dominant negative (DN) MsRop6^L GTPase (as baits). The growth of yeast colonies on selective drop-out media is shown. -Trp and -Leu stand for tryptophan- and leucin-free medium to select for the presence of bait and prey constructs within the same cells; -Ade and/or -His means the absence of adenine and/or histidine from the medium in order to select for bait-to-prey interaction. The MsRop6 GTPase forms were expressed in an isoprenylation defective form (MsRop6^L) to allow nuclear accumulation in the yeast cells. AD, activation domain and BD, DNA-binding domain. (B) In vitro pull down assay using purified 6 × HIS-tagged (HIS:MtRRK1) kinase as a prey and maltose-binding protein-tagged MsRop6 GTPase (MBP:MsRop6) in various forms (CA, DN and WT in the presence or absence of GTP or GDP, respectively) as a bait. Maltose-binding protein (MBP) and pure amylose resin (Resin) were used as controls. Proteins detected on the Western blots are indicated on the left, the used antibodies on the right. The relative amounts of the input proteins are shown above, while that of the pulled down kinase below, the line.

3. Results

3.1. Identification of MsRop6 GTPase-interacting kinases in the yeast two-hybrid system

In order to allow the identification of proteins interacting with the active or inactive MsRop6 GTPase in the yeast two-hybrid system, several MsRop6 mutants have been created. The $G_{15}V$ constitutive active mutation locks the GTPase in the GTP-bound conformation (MsRop6^{CA}) while the $T_{20}N$ dominant negative mutation reduces the rate of GDP-to-GTP exchange (MsRop6^{DN}). As the active Rop GTPases are predominantly membrane localized, nuclear accumulation of the GTPase in the yeast cells was facilitated by a further mutation ($C_{194}G$, designated as "localization" or L mutation) disrupting the C-terminal CAAX isoprenylation motif (MsRop6^{L}).

As we were especially interested in effector proteins that interact with the active MsRop6 GTPase, a standard yeast two-hybrid screen has been carried out using the double-mutant MsRop6^{CA,L} and a *M. truncatula* root-nodule cDNA library [16]. The *Medicago sativa* and *M. truncatula* Rop6 GTPases share 98.5% identity and 99.5% similarity at the amino acid level that allowed us to apply this interspecific screening approach [23].

The experiment resulted in the isolation of two cDNA clones coding for putative kinase proteins designated as *M. truncatula* Rop-interacting receptor-like kinase 1 and 2 (MtRRK1 and 2). Based on sequence homology search the two kinase proteins belong to the same family of receptor-like cytoplasmic kinases (RLCK Class VI [19,24]) and are homologous to the Arabidopsis proteins coded by the At2G18890 and At5G35960 genes, respectively (Fig. 1).

Based on the homology to the Arabidopsis proteins as well as on *M. truncatula* EST sequences, it could be established that the cDNA of the MtRRK1 kinase coded for the full length protein except the starting methionine while the MtRRK2 kinase was N-terminally truncated missing 12 amino acids. The full length cDNA clones (Accession numbers: FM886833 and FM886834, respectively) have been obtained by PCR.

In order to test the specificity of the interaction of the MtRRK1 and 2 proteins with the various conformations of the MsRop6 GTPase, pairwise yeast two-hybrid interaction assays were carried out between the kinases and the wild type (WT), constitutive active (CA; $G_{15}V$), and dominant negative (DN; $T_{20}N$) MsRop6 mutants carrying also the $C_{194}G$ (L) mutation. Fig. 2 shows the growth of the yeast cells transformed by the bait (MsRop6 forms fused to the DNA-binding domain, BD) and prey (MtRRK1 or MtRRK2 kinase fused to the activation domain, AD) constructs on various drop-out media. Selection on tryptophan- and leucine-free media ensured the presence of both constructs within the same cells while growth in the absence of histidine and/or adenine was used to indicate the interaction of the bait and prey proteins (for details, see Ref. [17]).

As shown in Fig. 2A, both kinases showed interaction with the CA and the WT form of the GTPase but not with the DN version. Moreover, it could be observed that increasing the strength of the selection for protein-protein interaction, using histidine- and adenine-free medium to monitor HIS and ADE2 expression in par-

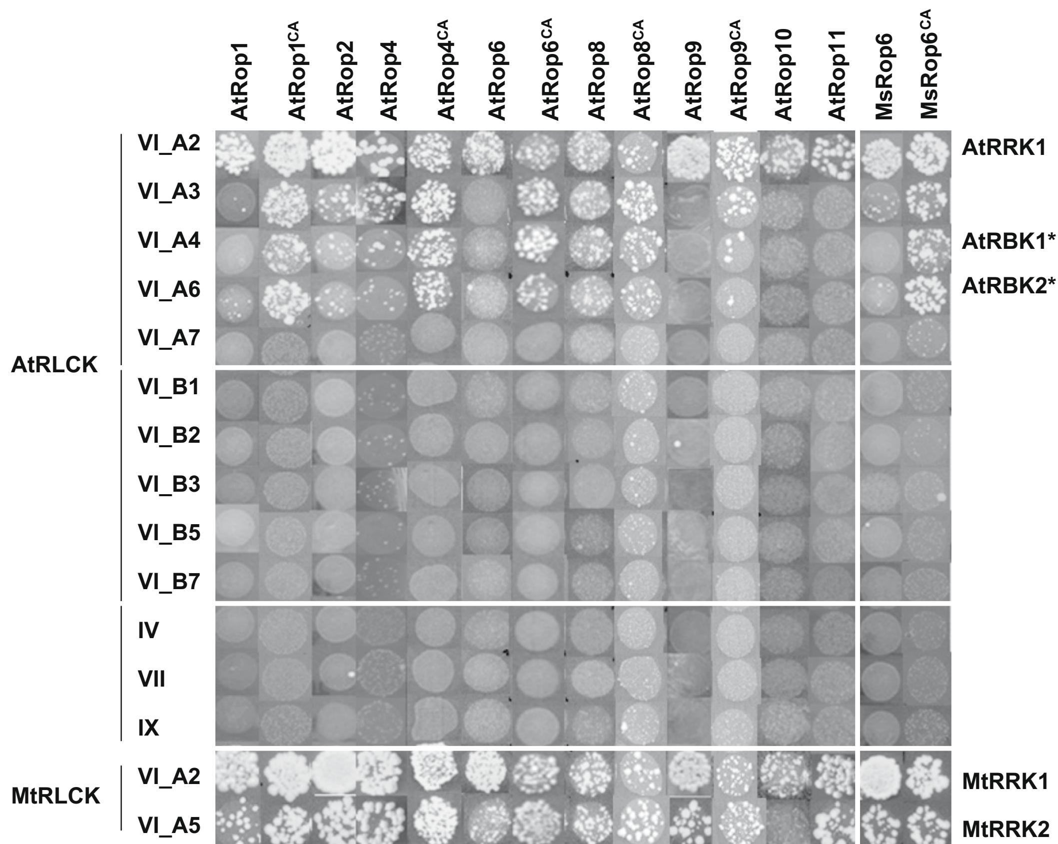

Fig. 3. Yeast two-hybrid interaction matrix of selected Arabidopsis and Medicago RLCK kinases and Rop GTPases. RLCK kinase and Rop GTPase genes were pairwise expressed in yeast cells as indicated. The growth of transformed yeast cells on Trp-, Leu-, His-drop-out medium is shown in the presence of 1 mM 3-aminotriazol to increase selection strength. Wild type and in some cases constitutive active (CA) Rop GTPases have been tested for the interaction. *AtRop4-binding RLCK kinases described by Molendijk et al. [29].

allel, the detection of the interaction was hampered in the case of the WT but not the CA GTPase (Fig. 2A).

The interaction of the MsRop6 GTPase and the MtRRK1 kinase was verified by an in vitro pull down assay using purified maltose-binding protein-tagged MsRop6 (MBP:Rop6) forms and the purified 6 × HIS-tagged MtRRK1 (HIS:RRK1) kinase (Fig. 2B). The binding of the kinase to all MsRop6 versions (WT, DN, CA, GTP- or GDP-bound) could be observed in this in vitro experiment (Fig. 2B). The difference between the in vitro and yeast two-hybrid interaction pattern might indicate that the active/inactive GTPase protein has different in vivo and in vitro conformation and/or preference for protein partners.

3.2. The specificity of Rop GTPase–RLCK VI kinase interaction

In order to test the specificity of Rop GTPase–RLCK kinase interaction, cDNA clones of eight different *A. thaliana* Rop GTPases, 10 Arabidopsis RLCK VI kinases (5 from group A, 5 from group B) and three further kinases belonging to other RLCK families (IV, VII, IX) have been inserted into yeast two-hybrid vectors. The MtRRK1 and MtRRK2 kinases and the MsRop6 GTPase were also included into the experiment. The yeast two-hybrid interaction matrix (Fig. 3) indicated that all but one investigated RLCK VI_A kinases (including MtRRK1 and 2) but none of the studied AtRLCK VI_B or AtRLCK IV, VII, or IX kinases showed interaction with the Arabidopsis or Medicago Rop GTPases involved into the experiment. The MtRRK1 kinase and its Arabidopsis homolog, AtRLCK VI_A2, could interact with all investigated Rop GTPases independent of their WT or CA conformation. The MtRRK2 kinase did not show interaction only with the AtRop10 GTPase (the cDNA clone of the Arabidopsis homolog of the MtRRK2 kinase could not be isolated and tested). Interactions of the other RLCK VI_A kinases could readily be detected only with the CA versions of the investigated GTPases.

3.3. RLCK VI kinases are specifically activated by active Rop GTPases in vitro

In order to test the ability of the MsRop6 GTPase to activate the MtRRK1 or MtRRK2 kinases, in vitro kinase assays were performed. As shown in Fig. 4, both kinases could very efficiently phosphorylate the myelin basic protein (MyBP) substrate in the presence of either the CA or the GTP-bound MsRop6 GTPase. MyBP phosphorylation was much weaker in the absence of GTP-bound MsRop6 GTPase, especially in the case of MtRRK1.

The phosphorylation of the CA or GTP-bound MsRop6 GTPase itself could also be observed. However, the phosphorylation site on the N-terminally HIS-tagged GTPase was found to be artificially formed during the cloning procedure (Supplementary material 2) and could not be observed in the case of a C-terminal 6 × HIS-tag (see also Fig. 5).

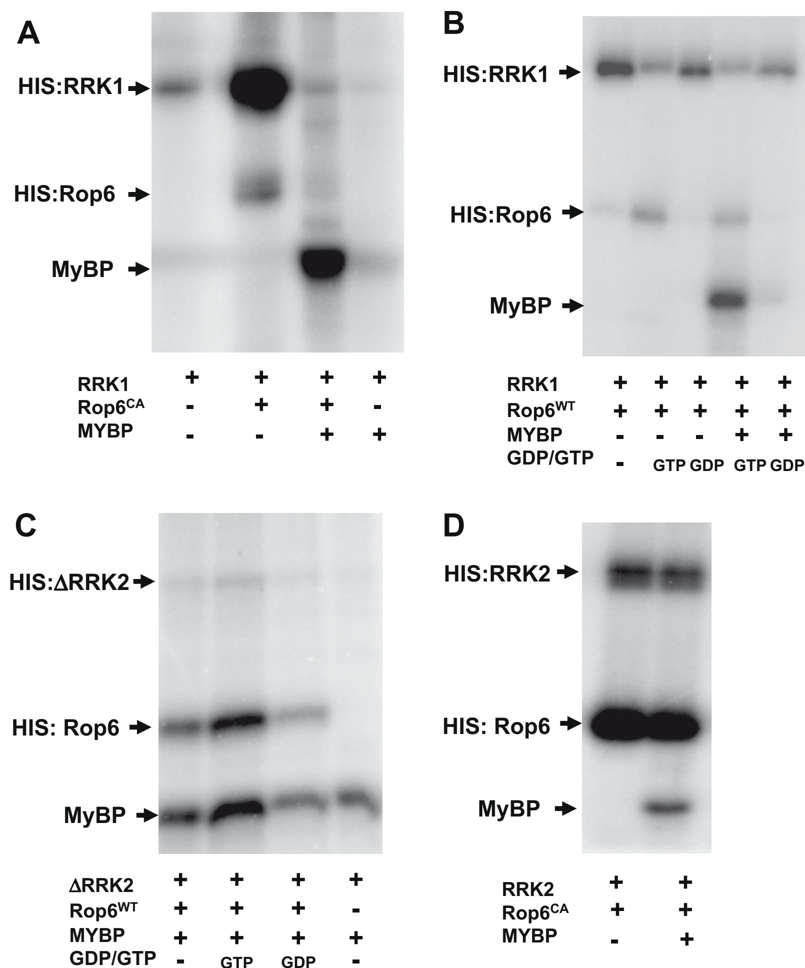

Fig. 4. In vitro activation of MtRRK kinases by the MsRop6 GTPase. In vitro activity of the MtRRK1 (A, B) and MtRRK2 (C, D) kinases in the presence or absence of MsRop6 GTPase in various forms (WT, wild type and CA, constitutive active). The WT GTPase was tested in the presence of GTP, GDP or none. In some of the reactions, myelin basic protein (MyBP) was incorporated as a kinase substrate. The positions of the HIS-tagged kinases, the HIS-tagged GTPase as well as the MyBP are indicated beside the autoradiograms. ΔRRK2 represents a truncated MtRRK2 kinase missing the first 12 N-terminal amino acids.

Autophosphorylation of the kinases was also detected (Fig. 4A and B). The autophosphorylated residues of the MtRRK1 kinase were determined to reside on the N-terminus (sites T_5 and S_7). N-terminal autophosphorylation of the MtRRK2 kinase was experimentally validated; a truncated form missing the 12 N-terminal amino acids (MKEKVDSPITVL) did not exhibit autophosphorylation in contrast to the full length version although it retained its activity (Fig. 4C and D).

Decreased autophosphorylation of the kinase and phosphorylation of the HIS:Rop6 GTPase could be observed in the presence of the myelin basic protein (MyBP) substrate indicating a preference for this artificial substrate under the reaction conditions (Fig. 4).

The activities of the Arabidopsis kinases AtRLCK VI_A2 and B3, representing the A and B groups of RLCK Class VI, and a further kinase belonging to RLCK Class VII have also been tested. Fig. 5A shows that both the MsRop6 and the AtRop1 GTPase could activate the AtRLCK VI_A2 kinase in the presence of GTP. However, the AtRLCK VI_B3 or the AtRLCK VII-type kinases could not be activated by the active MsRop6 GTPase in vitro (Fig. 5B).

3.4. Attempts to identify protein regions involved in the binding and activation of the MtRRK1 kinase by the MsRop6 GTPase

In order to limit the regions of the MtRRK1 protein involved in MsRop6 binding, N- as well as C-terminally truncated kinase versions were created (Fig. 6A) and cloned into yeast two-hybrid vectors. As shown in Fig. 6B, neither the 73, 174 and 331 amino acid-long N-terminal parts nor the 307 amino acid-long C-terminal part of the kinase were able to interact with the $MsRop6^{CA,L}$ GTPase in the yeast two-hybrid system. Only the 367 amino acid-long C-terminal part (missing 14 amino acids from the N-terminal end) preserved the capability for the interaction.

Considering the Rop GTPase, it was investigated whether the Rho-insert region (between amino acids 123–140 of MsRop6), implicated in the specificity of effector activation by Rho GTPases [25], has a role in RLCK binding and activation. The insert region of the MsRop6 GTPase has been replaced by a corresponding region of the human Ras GTPase as based on similar studies with human Rho-type GTPases [26,27]. The mutant $MsRop6\Delta I^{RAS}$ GTPase, both in WT,L and CA,L form, have been tested for interaction with the MtRRK1 and MtRRK2 kinases in the yeast two-hybrid system. As shown in Fig. 6C, the interaction was not prevented by the mutation as shown by the growth of the yeast colonies on selective media. However, as demonstrated in Fig. 6D, the $MsRop6\Delta I^{RAS,CA,L}$ GTPase version could not activate the MtRRK1 kinase in vitro indicating a role for the Rop insert region in RLCK activation.

4. Discussion

Despite of the central signaling role of Rho GTPase-activated kinases in fungi and metazoa, kinases directly activated by plant

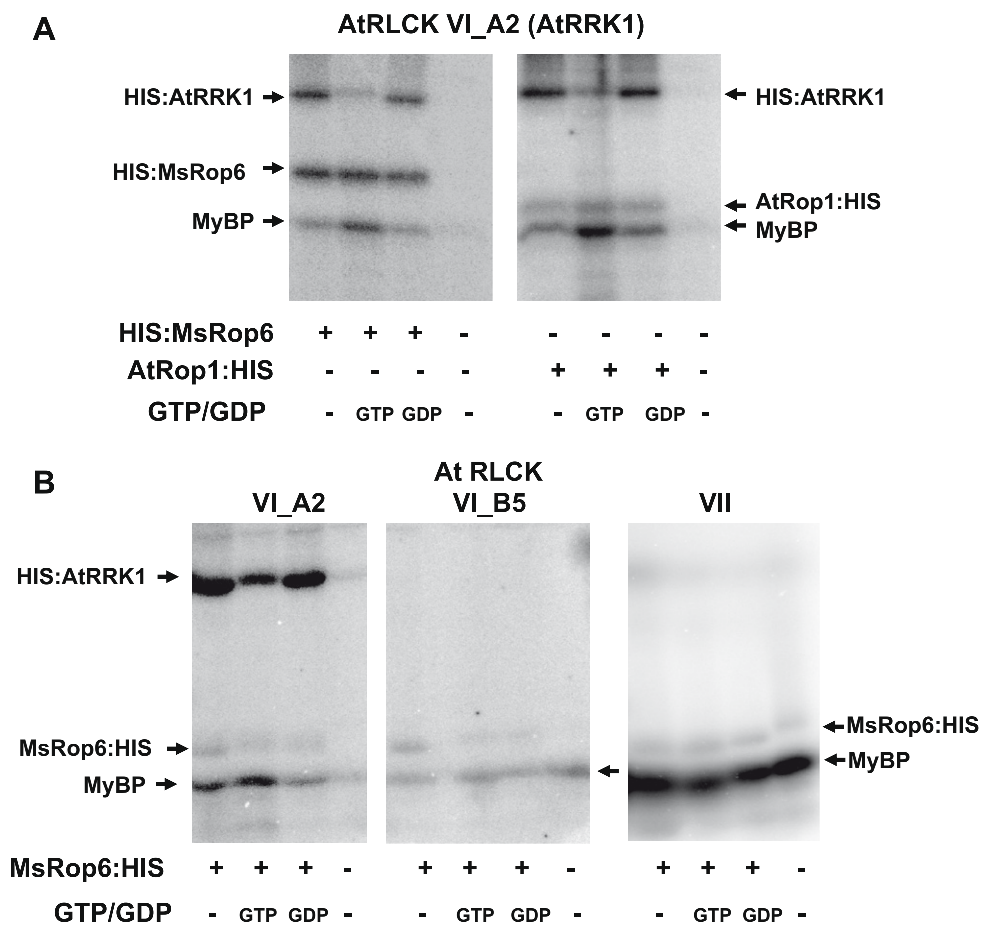

Fig. 5. In vitro activity of various Arabidopsis RLCK kinases in the presence and absence of Rop GTPases: (A) The in vitro myelin basic protein (MyBP) phosphorylating activity of the AtRLCK VI_A2 kinase (AtRRK1) could be increased by Medicago (MsRop6) as well as Arabidopsis (AtRop1) GTPases especially in the presence of GTP. The MsRop6 GTPase was N-terminally HIS-tagged and therefore could also be efficiently phosphorylated by the kinase in contrast to the C-terminally HIS-tagged AtRop1 (see Supplementary material 2). (B) In vitro MyBP phosphorylating activity of representing members of the RLCK VI group A (VI_A2 or AtRRK1), group B (VI_B5), and RLCK VII (VII) families in the presence or absence of the C-terminally HIS-tagged MsRop6 GTPase with or without GTP/GDP, as indicated. The position of the autophosphorylated HIS:AtRRK1 kinase is also indicated.

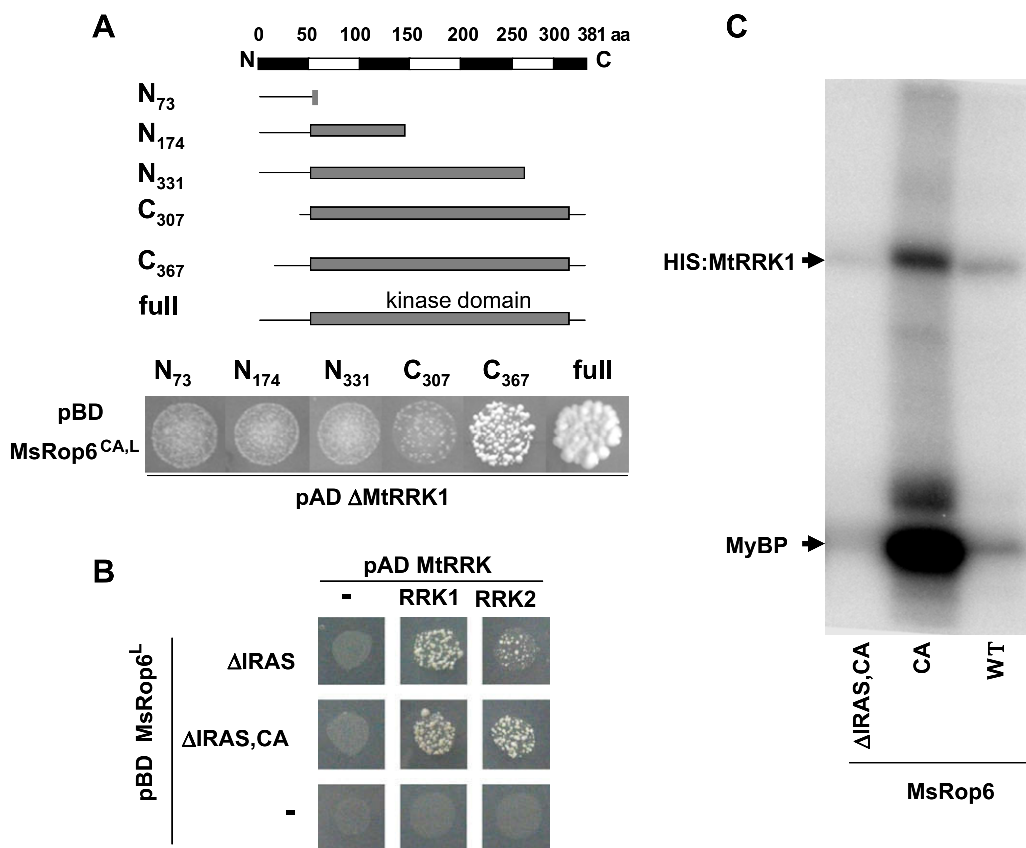

Fig. 6. Attempts to identify protein regions involved into the binding and activation of the MtRRK1 kinase by the MsRop6 GTPase. (A) Various N- and C-terminal deletions of the MtRRK1 kinase tested in a yeast two-hybrid interaction assay are schematically shown. N – N-terminal and C – C-terminal kinase fragment. Numbers indicate the length of the truncated protein in amino acids. Growth of yeast cells expressing the truncated and the full length (full) MtRRK1 kinase together with the constitutive active MsRop6 GTPase is shown on -Trp, -Leu, -His drop-out medium in the presence of 1 mM 3-aminotriazol to increase selection strength. pAD – activation domain plasmid and pBD – DNA-binding domain plasmid. (B) Yeast two-hybrid interaction of the MtRRK1 and two kinases with the MsRop6^{L} and MsRop6^{CA,L} GTPases where the Rho-insert region was exchanged for the appropriate region of the human Ras1 GTPase (ΔI^{Ras}). Yeast growth is shown as above. (C) In vitro MtRRK1 kinase activity assay in the presence of the MsRop6, MsRop^{CA} and MsRop6$^{\Delta IRAS,CA}$ GTPases and the myelin basic protein (MyBP) substrate.

Rho-type (Rop) GTPases could not be revealed in plants until now (for reviews [6,8,10]). There were, however, indirect indications that GTPase- and kinase-mediated signaling pathways are indeed interlinked in plants. Silencing of the small GTPase OsRac1 by RNA interference prevented the elicitor-induced accumulation and post-translational activation of the rice kinase OsMAPK6 [28]. However, the mechanism of OsRAC1-dependent OsMAPK6 activation is still not known. Recently, Molendijk et al. [29] found two Arabidopsis RLCK VI family members (RBK1 and RBK2) to bind directly the AtRop4 GTPase in the yeast two-hybrid system, in vitro and *in planta*.

In the present paper it is shown that two other plant RLCK VI family members, Medicago MtRRK1 and MtRRK2, can also directly interact with Rop GTPases. The yeast two-hybrid interaction matrix as well as in vitro kinase activation experiments with selected GTPase and kinase proteins confirmed that the interaction is specific for the group A [19] of the Arabidopsis RLCK VI family (with the exception of the AtRLCK VI_A7 kinase from presently unknown reasons). No Rop-binding could be observed in the case of randomly selected representants of the RLCK IV, VII and IX families using the yeast two-hybrid system. However, the recently published interaction of the AtRop4 and AtRop11 GTPases with an RLCK Class VIII kinase (NRCK [29]) suggests that the capability of RLCKs to bind Rop GTPases is not limited to the RLCK VI group A. Based on this observation and considering the high number of RLCK kinases in plants (ten families with 193 members in Arabidopsis) the existence of further Rop-binding RLCKs can be hypothesized.

Interestingly, none of the Rop-interacting RLCK kinases have any recognizable sequence motif known to play role in the binding of Rho-type GTPases to their effectors [7,9,30] and the Rop-binding ability could not be attributed to one well defined protein region (Fig. 6, [7]). The interaction of RLCKs with Rops may be based on a presently unknown mechanism and structural feature that needs further investigations. It maybe helpful in this respect that closely related sequences, such as of the members of the RLCK VI group A and group B, differ in their Rop-binding abilities.

The MtRRK1 and 2 as well as the investigated AtRLCK VI_A2 kinase have very low in vitro kinase activity under standard conditions. Their activity is strongly increased in the presence of active, GTP-bound, but not of inactive, GDP-bound, Rop GTPases supporting a hypothesis that these kinases are potential downstream Rop GTPase effectors. This possibility was disapproved by Molendijk et al. [29] as they could not observe Rop-GTPase-dependent in vitro autophosphorylation or AtRop4 substrate phosphorylation changes of NCRK:YFP and RBK:YFP kinases immunoprecipitated from insect cells. Our preliminary results, however, indicate that the RBK2 kinase can also be activated by Rop GTPases under the conditions described in the present paper (data not shown).

A specific region of Rho GTPases, the so called Rho-insert region, is implicated in effector binding and activation [7]. Plant Rop GTPases have an insert region with characteristic differences as compared to human or yeast Rac, Rho ad Cdc42 proteins that is an indication that plants have evolved specific Rop GTPase effectors [9]. Here evidence is provided that this region is important for

the activation of a plant-specific Rop effector, the RRK1 kinase. Although the described in vitro results highlight the possibility that certain RLCK kinases may function as Rop GTPase effectors in plants, further *in planta* experimentation is needed to verify this hypothesis. The fact that the expression pattern of many Rop-interacting RLCKs is linked to developmental processes (pollen tube growth, tracheary element differentiation, pathogen response etc. [19,29,31]), where the role of Rop GTPases has already been well established, is encouraging in this respect.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.02.047.

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