Available online at www.sciencedirect.com
ScienceDirect
Plant Physiology and Biochemistry 46 (2008) 380–393
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Review

Structure and function of Rho-type molecular switches in plants

Antje Berken*, Alfred Wittinghofer

Max Planck Institute of Molecular Physiology, Structural Biology Department, Otto Hahn Strasse 11, 44227 Dortmund, Germany

Received 28 September 2007
Available online 31 December 2007

Abstract

Molecular switches of the Rho family, in concert with their associated regulators and effectors are well known as important control elements of vital signaling pathways in eucaryotic organisms. Yet, this knowledge has so far been established mainly from animal and fungal studies. However, during the recent years, the Rho switch has gone increasingly green as well, and it turned out that the homologous system in plants holds some distinctive features regarding structures, functions and molecular mechanisms for signal transduction. In this review, we give an overview about the structural characteristics of the Rho proteins of plants, termed ROP, highlighting some exciting differences to their animal and fungal counterparts. We further address the unique regulators and effectors of the ROPs and discuss the structural basis for the function and interaction of those proteins in ROP controlled reaction cascades. We finally intend to stimulate the demand for future three-dimensional structures that advance our understanding of the ROP switch in plants.

Keywords: Rho of plants; ROP/RAC; Three-dimensional structure; Rho insert; RopGEF; RopGAP; Effector

1. Introduction

Rho-type molecular switches make up a family of small guanine nucleotide binding proteins, short G proteins, which, together with Ras, Ran, Arf and Rab proteins constitute the Ras superfamily with evolutionary conserved orthologues in most eucaryotic organisms [23,89,102]. These small G proteins bind GDP and GTP with high affinities and can slowly hydrolyze GTP [15,96]. Based on the latter activity, the proteins are also known as GTPases although they are, as such, pretty poor catalysts. G proteins switch between their active GTP-bound conformation and the inactive GDP-bound conformation by strictly regulated nucleotide exchange and GTP hydrolysis. Activation is catalyzed by guanine nucleotide exchange factors (GEFs), and GTPase activating proteins (GAPs) promote GTP cleavage to return the switch back to the inactive GDP-state. Only the active GTP-form is able to interact with downstream effectors to exert its biological function. That way, the Ran, Arf and Rab proteins control nuclear and vesicular trafficking processes in eucaryotic cells while the Rho and the Ras family members regulate extracellular stimulus-dependent signaling pathways that affect gene

Abbreviations: ACK, activated Cdc42 associated kinase; Arf, ADP ribosylation factor; Arp, actin-related protein; *At*, *Arabidopsis thaliana*; BIG, brefeldin A-inhibited guanine nucleotide exchange protein; CCR, cinnamoyl-CoA reductase; Cdc42, cell division cycle 42; CRIB, Cdc42/Rac interactive binding; CZH, CDM-Zizimin-homology; Dbl, diffuse B-cell lymphoma; FragX-IP, Cytoplasmic fragile X mental retardation protein interacting protein; GAP, GTPase activating protein; GBD, G protein binding domain; GBF, Golgi-specific brefeldin A resistance factor; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; HVR, hypervariable region; ICR, interactor of constitutive active ROPs; KPP, kinase partner protein; PAK, p21-activated kinase; PH, pleckstrin homology; PIR, p53 inducible RNA; P-loop, phosphate binding loop; PM, plasma membrane; PNK, polynucleotide kinase; PRK, pollen-specific receptor kinase; PRONE, plant-specific ROP nucleotide exchanger; PRR, proline-rich region; Rab, Ras-like protein in brain; Rac/RAC, Ras-related C3 botulinum toxin substrate; Ran, Ras-like nuclear; Ras, rat sarcoma; Rho, Ras homologue; RIC, ROP-interacting CRIB-motif containing protein; RLK, receptor-like kinase; ROCK, Rho associated kinase; ROP, Rho of plants; SRA, specifically Rac associated; UGT/UDPGT, UDP-glucose transferase; VCA, Verprolin/Central/Acidic; WASP, Wiskott-Aldrich syndrome protein; WAVE, WASP family verprolin homologous protein; WHD, WAVE homology domain; 3-D structure, three-dimensional structure.

* Corresponding author. Tel.: +49 231 133 2103; fax: +49 231 133 2699.
E-mail address: antje.berken@mpi-dortmund.mpg.de (A. Berken).

0981-9428/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2007.12.008

expression, cell proliferation, differentiation and survival in the case of Ras, and actin reorganization, cell cycle progression, cell polarity and the expression of further genes in case of the Rho proteins. Ras mediated signal transduction is indispensable for proper cell functioning in animals and fungi. Plants, however, seem to lack true Ras proteins so that the Rho family has got into the center of attention for small G protein regulated signaling events [7,97,109]. The Rho family itself can be further grouped into discrete subfamilies comprising the major classes Rho, Rac and Cdc42 that are generally found in animals and fungi, but astonishingly not in plants [14,97,111]. Instead, the plants have apparently evolved a unique class of Rho proteins which, according to today's predominant nomenclature, is mostly referred to as ROP, for Rho of plants [7,109,111].

2. ROP – a unique Rho subfamily in plants with diverged subgroups

ROP proteins are thought to be ubiquitously present among embryophytes and have yet been identified in mosses, conifers and both monocot and dicot flowering plants [22,103]. In most species analyzed so far, the ROPs are encoded by a multigene family and gene duplications likely happened during evolution [103]. The by far best characterized proteins are probably the eleven ROPs from the model plant *Arabidopsis thaliana* (*At*ROP1–*At*ROP11) [61,103]. They share 70–98% amino acid identity with each other and at least 45% identity with the other members of the Rho family being most similar to the mammalian Rac proteins with up to 61% identical residues with human Rac1 [103,111]. Due to this high overall similarity to Rac, the ROPs have also been denoted as plant RAC proteins, and a recent evolutionary analysis of the Rho family in eucaryotes revealed that Rac genes are probably the founders of the ROP but also of the Cdc42 subfamily [14]. Nevertheless, this study differentiates between the ROP, Rac, Cdc42 and Rho clades which all constitute clearly identified clusters supporting the opinion that the ROPs represent a unique Rho subfamily that is specific for plants [2,111].

As the sole representatives of the Rho family the ROPs are expected to cover a vast spectrum of Rho functions in plant cells and thus may have structurally diverged to allow interactions with diverse cellular targets. Two major phylogenetic ROP subgroups, termed type-I and type-II, have initially been described whose members differ mainly in their gene structures and C-terminal amino acid sequences [103]. In *Arabidopsis* *At*ROP1 to *At*ROP8 belong to the type-I group while *At*ROP9, 10 and 11 were classified as type-II ROPs. This two-type-classification is reflected in distinct posttranslational lipid modifications that are necessary for interactions of the proteins with membranes (see below). On the other hand, four distinct ROP groups (I–IV) have been suggested [111], and a more recent phylogenetic analysis including further monocot sequences corroborated the quartering but with some modifications resulting in four classes designated group 1–4 [22]. So far, it remains to be determined if and how subgroup-specific features go along with a probable 'neo-functionalization' within the ROP subfamily as it has recently been proposed for the Arf-GEF family GBF [90]. GBF proteins together with the BIG-type Arf-GEFs regulate endosomal trafficking and post-Golgi sorting in plants whereas eight Arf-GEF families are in charge to control those complex processes in other eucaryotic kingdoms [24,69]. Yet, this lack of multiple families in plants could get compensated by the diversity within the GBF family whose individual members may have evolved to acquire distinct novel functions [90].

3. Structure of the ROPs – the characteristics of *bona fide* Rho proteins

Like most Rho proteins in animals and fungi, the ROPs comprise about 200 amino acids with a molecular weight of approximately 21–24 kDa. Their prominent structural element is the G domain (Fig. 1A) which the ROPs have in common with all guanine nucleotide binding proteins [15,96]. It contains five highly conserved sequence motifs, called G-box-motifs (G1–G5) which are essential for binding of the nucleotide and the associated Mg^{2+} ion, and for GTP hydrolysis [15,96,102]. Interactions usually occur between the guanosine moiety of the nucleotide and amino acids of the G4 and G5 region while residues of the G1-motif make a tight contact to the α- and β-phosphates. When GTP is bound to the G domain, the γ-phosphate is contacted by key residues of the G2 and G3 regions, and those also mediate the binding of Mg^{2+}. G3 contains the catalytic glutamine residue which positions the nucleophilic water molecule for GTP hydrolysis. Consensus sequences for those G-box-motifs in the plant proteins (Fig. 1A) have been deduced from a large scale comparison of ROP proteins from multiple monocot and dicot species [22], and the respective amino acids represent variations which are in good agreement with the consensus motifs originally described by Bourne and colleagues [15]. Frequently used mutations that generate constitutively-active (CA) or dominant-negative (DN) ROP forms [109] also affect key residues within those G-box-motifs (Fig. 1A). Based on what is known for the homologous mutations in Ras [33,63], it is believed that substitutions of an invariant glycine in G1 (G15 in ROP9) or the glutamine in G3 (Q64 in ROP9) interfere with GTP hydrolysis thus keeping the ROPs in the active state. Mutating the consensus threonine in G1 (T20 in ROP9) or the aspartate in G4 (D121 in ROP9) is thought to result in reduced nucleotide and increased GEF affinities. Those mutant proteins apparently compete in cells with normal ROPs for binding to their GEFs and consequently act as dominant-negative homologues of the wildtype proteins.

Three-dimensional structure information on the ROP switch is so far limited to two proteins from *Arabidopsis*, the type-II *At*ROP9 in its free form bound to GDP (PDB code 2J0V [86]) and the type-I *At*ROP4 in complex with GDP and the catalytic domain PRONE (plant-specific ROP nucleotide exchanger) of its activating GEF (PDB code 2NTY [92]). Both models show a G domain with the typical topology [72,96,102]. It expectedly folds into a globular molecule composed of a central β-sheet with 6 strands (β1–β6)