Review

Spatial control of Rho (Rac-Rop) signaling in tip-growing plant cells

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Spatially restricted signaling by Rho GTPases is essential for the polarization of eukaryotic cells, which is required for the morphogenesis, mobility and division of single cells, and for the development of multicellular organisms. Rac-Rop GTPases, which constitute a plant-specific Rho GTPase subfamily, accumulate at the apical plasma membrane of pollen tubes and root hairs, where they control rapid polar cell expansion by a process known as tip growth. Here, recent insights into the spatial control of Rac-Rop-dependent signaling in tip-growing plant cells by regulatory proteins (i.e. Rho GTPase-activating proteins, Rho guanine nucleotide dissociation inhibitors, Rho guanine nucleotide-exchange factors and phosphoinositide-specific phospholipase C) and lipids [phosphatidylinositol (4,5)-bisphosphate and diacyl glycerol] are summarized. A model is presented, which integrates the current knowledge concerning the molecular mechanisms that maintain the polarization of Rho signaling in plant cells.

Introduction

Small GTPases of the Rho family are important signaling proteins found in all eukaryotic cells that regulate and coordinate a variety of cellular processes, including reorganization of the actin cytoskeleton and membrane trafficking [1]. The large animal Rho GTPase family comprises Rho, Rac and Cdc42 proteins, whereas yeast cells exclusively express Rho and Cdc42 isoforms [2]. In plants, Rho GTPases are represented by a much smaller subfamily of proteins that are most closely related to animal Rac and are referred to as Rac-Rop (Rho of plant) GTPases [3,4]. Spatially restricted signaling based on the accumulation and activation of Rho-family GTPases at selected plasma membrane domains has a key role in the polarization of eukaryotic cells, and is required for the directional expansion, motility, asymmetric division and differentiation of various cell types. Processes depending on polarized Rho-GTPase signaling include: bud formation, bud growth [5] and directional cell expansion [6] in yeast; elongation of neuronal axons [7]; tip growth of fungal hyphae [8] and plant cells [9]; morphogenetic cell-shape changes in *Drosophila* embryos [10]; migration of animal and *Dictyostelium* cells [11,12]; asymmetric division of *Caenorhabditis elegans* zygotes [13]; and junction formation between polarized epidermal cells [14].

Here, the recent findings concerning the molecular mechanisms that locally restrict Rho (Rac-Rop) signaling at the tip of pollen tubes and root hairs are integrated into a coherent model, which is put in context with the current knowledge of the spatial control of Rho signaling in other systems. A discussion of other aspects of Rho signaling (e.g. developmental or temporal regulation of Rho activity and Rho-dependent signaling cascades), the research of which has also progressed greatly during the past few years, is, however, beyond the scope of this review.

Molecular mechanisms that polarize Rho, Rac and Cdc42 signaling

Although different biochemical activities of Rho GTPase are known to be controlled by a conserved group of regulatory proteins, with which they directly interact (Box 1), the molecular mechanisms responsible for polarized Rho, Rac and Cdc42 accumulation, activation and signaling in intact cells are not well understood [15]. The spatial control of Cdc42-dependent signaling has been particularly intensely studied in the budding yeast *Saccharomyces cerevisiae*. Yeast bud formation and growth requires polarized accumulation of activated Cdc42 at plasma membrane domains, which can be specified by the presence of bud-site-determination cues. Polarization of Cdc42 activity during this process depends on two different positive-feedback loops [5]: Cdc42 activity at the tip of forming and growing yeast buds self-enhances (i) by promoting further CDC42 recruitment to the site by stimulating actin-based transport [16], and (ii) by recruiting Cdc24, a Rho guanine nucleotide-exchange factor (RhoGEF) that activates Cdc42, via the promotion of conformational changes in the scaffolding protein Bem1p [17].

RhoGEFs (Box 1) also specifically accumulate at the plasma membrane at sites of Rho GTPase activation in the fission yeast *Schizosaccharomyces pombe* [6] and in animal cells [10,18], where they are thought to have key roles in the spatial control of Rho GTPase signaling [19]. The functions of Rho GTPase-activating proteins (RhoGAPs) and Rho guanine nucleotide dissociation inhibitors (RhoGDIs) (Box 1) in this process are less well understood. Interestingly, the absence of a RhoGDI has been reported to reduce the Cdc42-dependent polarized growth of fungal hyphae [8], indicating that RhoGDI activity is required for this process. Moreover, the distribution of a RhoGAP was recently found to be complementary to Rho activity at the plasma membrane of polarized epithelial cells in *Drosophila* embryos [10]. Downregulation of RhoGAP activity by cyclin-dependent kinase (CDK)-mediated phosphorylation is required for cell-cycle controlled Cdc42 activation and cellular polarization in yeast [20–22]. RhoGAP phosphoryl-

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Box 1. Regulation of Rho activity by proteins that directly interact with Rho GTPases

Most Rho GTPases are post-translationally modified by C-terminal prenylation, which is responsible for their association with the plasma membrane. Rho GTPases are active as signaling molecules in the GTP-bound conformation and interact with multiple effectors in this state (Figure I).

GTPase-activating proteins (RhoGAPs) inactivate the signaling function of Rho GTPases by stimulating their low intrinsic GTPase activity, which converts them to the inactive GDP-bound conformation (brown; Figure I). Animal cells express many RhoGAPs, which contain a variety of different functional domains in addition to the conserved domain that is responsible for RhoGAP activity [68]. In plants, RhoGAPs form a much smaller family of proteins, which exhibit little homology to their animal counterparts outside of the conserved RhoGAP domain [69,70]. Plant RhoGAPs involved in the control of tip growth (see Figure 2a in main text) contain a CRIB (Cdc42-Rac interactive binding) domain directly adjacent to the RhoGAP domain. Interestingly, CRIB domains are not found in animal RhoGAPs but they mediate the specific interaction of many effectors with activated forms of Rho GTPases in all eukaryotic cells [71,72].

Guanine nucleotide-exchange factors (RhoGEFs) are usually associated with the plasma membrane and activate the signaling function of Rho GTPases by promoting the exchange of GDP for GTP (Figure I). The number of different RhoGEFs expressed in animal cells approximately equals that of RhoGAPs. Animal RhoGEFs belong to two structurally completely unrelated protein families [73,74]. With the possible exception of the *Arabidopsis* protein SPIKE1, which shows some homology to the animal DOCK-180 family of RhoGEFs and is required for normal cell-shape development [75], there are no plant homologs of animal RhoGEFs. Recently, a small family of plant proteins has been identified that contains a conserved PRONE (plant-specific Rop nucleotide exchanger domain conferring Rac-Rop-specific RhoGEF activity [47,48,64]. Homologs of these proteins do not exist in animals or in fungi.

A key biochemical activity of guanine nucleotide dissociation inhibitors (RhoGDIs) is removal of prenylated Rho GTPases from the plasma membrane and formation of heterodimers with Rho GTPases in the cytoplasm (Figure I). Consequently, RhoGDIs are most commonly seen as negative regulators of Rho GTPases [1], despite recent indications of their possible function in promoting Rho activation [8,45,46]. Animals and plants express only a few RhoGDIs isoforms, the structures of which are all closely related.

Several structurally unrelated membrane-associated proteins and membrane lipids, including phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], have been shown to destabilize interactions between animal Rho GTPases and RhoGDIs. These proteins and lipids might function as RhoGDI-displacement factors (RhoGDFs), promoting the re-association of Rho GTPases with the plasma membrane and, thus, the subsequent activation of Rho GTPases by RhoGEFs [43,76] (Figure I). Factors that destabilize interactions of Rac-Rop GTPases with plant RhoGDI isoforms have not been biochemically identified, but PtdIns(4,5)P_2 co-localizes with active Rac-Rop GTPases at the apex of tip-growing plant cells, where it might exert a dual function as a Rac-Rop effector and a RhoGDF (see Figure 2b in the main text).

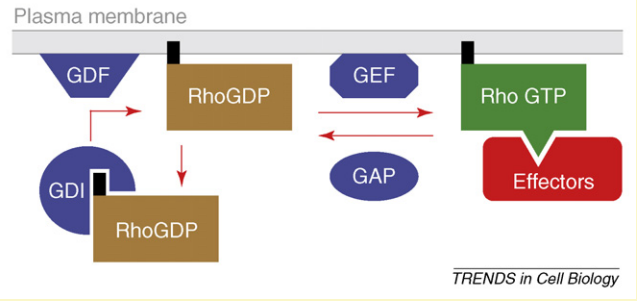

Figure I. Regulation of Rho activity by proteins that directly interact with Rho GTPases. Schematic representation of the control of Rho activity by conserved regulatory proteins (blue). Black rectangles represent the C-terminal prenyl tail.

ation might control yeast Cdc42 activity not only temporally in a cell-cycle dependent manner but also spatially at specific plasma membrane domains [20], although this remains to be demonstrated.

The membrane lipid phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] co-localizes with active Rac at the leading edge of migrating animal and *Dictyostelium* cells. PtdIns(3,4,5)P_3 can recruit and activate RhoGEFs that stimulate Rac activity, which, in turn, promotes PtdIns(3,4,5)P_3 production by lipid kinases. This positive-feedback mechanism has been proposed to enhance the polarization of Rac signaling in migrating cells [11,12]. Maintenance of polarized Rac signaling in these cells also seems to depend on the PtdIns(3,4,5)P_3 phosphatase PTEN, which is associated with the plasma membrane with a distribution pattern complementary to that of PtdIns(3,4,5)P_3 [23,24]. These findings indicate that the spatial control of Rac signaling during cell migration depends both on the control of the local activities of regulatory proteins (i.e. RhoGEFs) that directly bind to this Rho GTPase and on the molecular mechanisms that maintain polarized lipid distribution in the plasma membrane.

Investigation into the regulation of Rho, Rac and Cdc42 has revealed that several different molecular mechanisms are involved in the spatial control of Rho GTPase signaling. Although in most cell types only one or two of these mechanisms have been, to date, reported to be active, polarized Rho GTPase signaling in any cell type is likely to depend on regulation at multiple levels. It is becoming increasingly clear that the establishment and maintenance of polarized Rho GTPase signaling must be a dynamic process [25]. Plasma membrane domains at which active Rho GTPases accumulate, presumably, undergo constant remodeling as a consequence of the stimulation of membrane trafficking (i.e. secretion and endocytosis) by these proteins. Rather than the stable association of polarity determination factors with such membrane domains, dynamic interactions between multiple regulatory mechanisms, including positive-feedback loops, are therefore likely to spatially control Rho GTPase signaling.

Rac-Rop GTPases regulate tip growth

Root hairs and vegetative pollen tube cells are uni-axial, have an extremely elongated morphology and rapidly expand exclusively at one end by a process known as tip growth (Box 2). *Arabidopsis* root hairs are formed as a single outgrowth at the basal end of root epidermal cells (trichoblasts). They elongate at a rate of 1–2 μm min^{-1}, which is similar to the growth rate of neuronal axons [26], and can reach a length that exceeds their diameter (6–10 μm) >100 times [27]. Pollen tubes formed by germinating pollen grains on the stigma of mature flowers elongate markedly faster than roots hairs and can reach a length/diameter ratio of several thousand times [28]. Tobacco, lily and petunia pollen tubes – which, in contrast to *Arabidopsis* pollen tubes, grow in culture long enough to enable transient gene expression – are widely used as model systems for investigating the mechanism and regulation of tip growth. Cultured tobacco pollen tubes have a diameter of ~10 μm, elongate at an average rate of