Minireview

Signaling to the Rho GTPases: networking with the DH domain

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Received 25 October 2001; revised 17 December 2001; accepted 17 December 2001

First published online 4 January 2002

Edited by Gianni Cesareni and Mario Gimona

Abstract The Dbl homology (DH) domain was first identified in the Dbl oncogene product as the limit region required for mediating guanine nucleotide exchange on the Rho family GTPase Cdc42. Since the initial biochemical characterization of the DH domain, this conserved motif has been identified in a large family of proteins. In each case, a pleckstrin homology (PH) domain immediately follows the DH domain and this tandem DH–PH module is the signature motif of the Dbl family of guanine nucleotide exchange factors (GEFs). Recent structural studies have provided significant insight into the molecular basis of guanine nucleotide exchange by Dbl family GEFs, opening the door for understanding the specificity of the DH/GTPase interaction as well as providing a starting point for understanding how the exchange activity of these proteins is modulated to achieve specific biological outcomes in the cell. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Dbl; GEF; DH; Guanine nucleotide exchange factor

1. Introduction

Members of the Rho family of GTPases are remarkable in their ability to regulate a wide range of cellular responses, including reorganization of the actin cytoskeleton, gene expression, apoptosis, membrane trafficking events, mitogenic signaling, and malignant transformation [1–3]. The ability to bind and hydrolyze GTP lies at the heart of the biological activity of this family of proteins, allowing them to function as molecular switches cycling between an active, GTP-bound state and an inactive, GDP-bound state. Turning on the molecular switch requires the displacement of the bound GDP from the nucleotide-binding pocket and its subsequent replacement with cytosolic GTP, an event that is catalyzed by the Dbl family of guanine nucleotide exchange factors (GEFs) [4,5]. Along with other regulatory factors including GAPs and GDIs [6], the Dbl family of exchange factors provides exquisite control over the signaling events mediated by Rho family GTPases. Proteins of the Dbl family are defined by the presence of a conserved domain of ~150 amino acids, designated the Dbl homology (DH) domain, which is the limit region required for GEF activity. The DH domain is invariably coupled to a pleckstrin homology (PH) domain that is essential for the cellular function of many Dbl family proteins. Recently, significant progress has been made toward understanding the structural basis of the ability of Dbl family proteins to serve as GEFs toward Rho family GTPases. As described in the following sections, this structural information provides a context for understanding the cellular function of the Dbl family of exchange factors.

2. The DH domain defines a family of proto-oncogenes

The Dbl oncogene was first identified in screens designed to isolate transforming factors from a human diffuse B-cell lymphoma [7]. Oncogenic activation of Dbl occurs through an amino-terminal truncation of the 115 kDa proto-Dbl product. Sequence analysis revealed a region of oncogenic Dbl with significant similarity to the yeast Cdc24 protein [8]. Cdc24 was known to play a critical role in the signaling pathways leading to bud-site assembly in yeast and, based on genetic studies, was thought to lie upstream of another protein important in bud-site formation, the Rho family GTP-binding protein Cdc42 [9]. Given the sequence similarity between Cdc24 and Dbl, it seemed plausible that Dbl might play a role in regulating the cellular activity of the human Cdc42 protein, which led to the biochemical demonstration that oncogenic Dbl acts as a guanine nucleotide exchange factor (GEF) toward Cdc42 [10]. In addition to identifying Dbl as an upstream activator of Cdc42, these findings provided an important clue that misregulation of Cdc42-mediated signaling events is involved in oncogenic transformation.

These initial observations led to the identification of a large number of proteins sharing a tandem arrangement of DH and PH domains, thus making them candidate GEFs for Rho family GTP-binding proteins. Many of these proteins have been discovered on the basis of their transforming activity or are known to be involved in cell growth regulation, making the Dbl family one of the largest groups of proto-oncogenes. The sequence homology exhibited by Dbl family members within the DH domain is clustered in three highly conserved regions (CRs), and mutations within these regions compromise their GEF activity. Within the Dbl family, DH domains exhibit varying degrees of specificity with some members like Dbl acting on a number of Rho proteins while others such as p115, Tiam1 and intersectin show strong specificity for a single GTPase (Rho, Rac, and Cdc42, respectively). The fact that the DH and PH modules are invariably coupled in members of the Dbl family would seem to be indicative of a conserved function for the PH domain. However, the precise role of this domain remains unclear. The PH domain is not required for

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0014-5793/02/$22.00 © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(01)03310-5

the GEF activity of the DH domain but is essential for the transforming activity of many oncogenic Dbl family members [11]. PH domains are recognized to bind phosphoinositides [12] and may serve to target the GEF to its proper cellular location or participate in regulatory interactions that influence the functional activity of the DH domain (see below). In addition to the conserved DH and PH domains, Dbl family members are typically characterized by the presence of other signaling modules (Fig. 1) thought to couple their GEF activity to specific upstream signaling events that ultimately lead to the activation of Rho family GTP-binding proteins. The expectation is that as high resolution structural information becomes available for the different modules found in Dbl family proteins, particularly in complex with relevant binding or regulatory proteins, important insights will emerge regarding the mechanism of GEF activity, and how this activity is influenced by upstream signaling factors.

3. Architecture of the DH domain

The structures of five different DH domains are now available, providing important information about the basic structural features of the DH domain, as well as how this domain couples to Rho family substrates. The isolated DH domains of Trio [13] and Cool-1/βPIX [14] provide a picture of the basic architecture of the DH domain, while the intact DH–PH module of SOS [15], the complex of the DH–PH module of Tiam1 with Rac [16], and the auto-inhibited form of the Vav DH domain [17], provide snapshots of the DH domain in various signaling contexts. In each of these structures, the DH domain adopts an elongated structure with a unique all helical fold. The three regions of primary sequence that define the DH domain, conserved regions 1, 2, and 3 (noted CR1–3), each form a long α-helix and these three helices pack together forming the core of the DH domain. On one face, CR1 and CR3 contribute to the only significantly conserved solvent-exposed surface of the DH domain. Mutations that compromise the GEF activity of various DH domains map to this conserved surface. Opposite this conserved face, additional helices pack against the CR helices such that the overall secondary structure of the DH domain is roughly organized in five segments that resemble a five-helix bundle.

4. Mechanism of DH domain-mediated exchange

Thus far, perhaps the most significant advance in understanding the mechanism underlying the GEF activity of the Dbl proteins has been the determination of the X-ray crystal structure of Rac in complex with the DH–PH module of Tiam1 (Fig. 2) [16]. The conserved face of the DH domain formed by CR1 and CR3 of Tiam1 plays a primary role in forming contacts important for GEF activity. Specifically, the entire switch I region of Rac is shifted laterally along the nucleotide-binding cleft and lies in a groove between the CR1 and CR3 helices. Switch II forms even more extensive interactions with the conserved face of the DH domain including contacts with CR3 that induce conformational changes between residues 59 and 64 in Rac. A highly conserved glutamic acid residue in CR1 (Glu1047) forms important hydrogen-bonding contacts to the main chain amides of Thr35 and Val36 as well as to the side chain hydroxyl of Tyr32 in switch I of Rac. These interactions have the collective effect of destabilizing nucleotide binding by moving Thr35 out of the coordination sphere of the Mg^{2+} ion. The Mg^{2+} ion-binding site is further disrupted by the conformational change in switch II, which is reconfigured into two β-turns, similar to the conformational changes induced in switch II of Ras by the SOS RasGEF domain [18]. Importantly, the side chain methyl group of Ala59 from switch II of Rac is forced into the nucleotide-binding pocket where it sterically blocks the Mg^{2+}-binding site. Additionally, a glutamic acid residue (Glu62) supplied in *cis* from the switch II region of Rac contacts Lys16 in the P-loop, destabilizing the interaction of the GTPase with the β-phosphate of the nucleotide. These changes in switch II conformation are supported by interactions with a conserved lysine (Lys1195) from CR3 in the DH domain of Tiam1. The Mg^{2+} ion and P-loop form two critical contacts with the nucleotide and the Tiam1 DH domain mediates exchange by disrupting both of these interactions.

Distinct classes of exchange factors activate each family of Ras-like GTPases. These GEFs are structurally diverse, but have converged on a conserved mechanism for nucleotide exchange. This general mechanism, most clearly articulated by Wittinghofer and colleagues in relation to the Ran/RCC1 complex [19], involves disruption of Mg^{2+} ion binding and collapse of the P-loop. In addition to these common features, Tiam1 is unique in that the conformational changes in switch I bring Ile33 into steric clash with the ribose, facilitating ejection of the nucleotide. The conserved nature of the residues involved in disruption of the Mg^{2+} ion-binding site and P-loop of Rac by Tiam1 suggest that similar interactions are central to the exchange mechanism of all DH domains.

The fundamental nature of these conserved residues to the mechanism of nucleotide exchange is emphasized by the crystal structure of the Cdc42/GDI complex [20] in which the GDI engages a similar set of residues on the GTPase to stabilize Mg^{2+} binding and exert an opposing biochemical effect (i.e. inhibiting rather than stimulating the dissociation of GDP). Mutation of the residues in the DH domain responsible for disruption of the Mg^{2+} ion-binding site in the Rac/Tiam1 complex (Glu1047 and Lys1195 of Tiam1) compromises or completely blocks GEF activity in a variety of Dbl family proteins. Changing the conserved glutamic acid residue in CR1 of the Trio DH domain to alanine significantly slows the rate of nucleotide exchange [13], and the equivalent mutation in the DH domain of Dbl almost completely blocks its GEF activity [21]. This glutamic acid residue is highly conserved, with a few notable exceptions, including an alanine at the equivalent position of SOS, a threonine in RasGRF, and a glycine in both ABR and BCR. This lack of conservation is likely to have consequences on the nucleotide exchange activity of these DH domains, although this has yet to be investigated. Similarly, most DH domains contain a lysine or arginine at the position corresponding to Lys1195 in the CR3 domain of Tiam1. Two notable exceptions are Lbc and Lfc, which have hydrophobic residues at this position (Val and Leu respectively). In vitro exchange assays for both Lbc and Lfc show that these proteins are highly specific GEFs for Rho and do not act on Cdc42 or Rac. It will be of interest to understand how these GEFs are able to function while lacking this critical lysine and if these differences are important to their specificity.

A significant portion of the complex interface is mediated by a patch of highly variable sequence in the DH domain on a

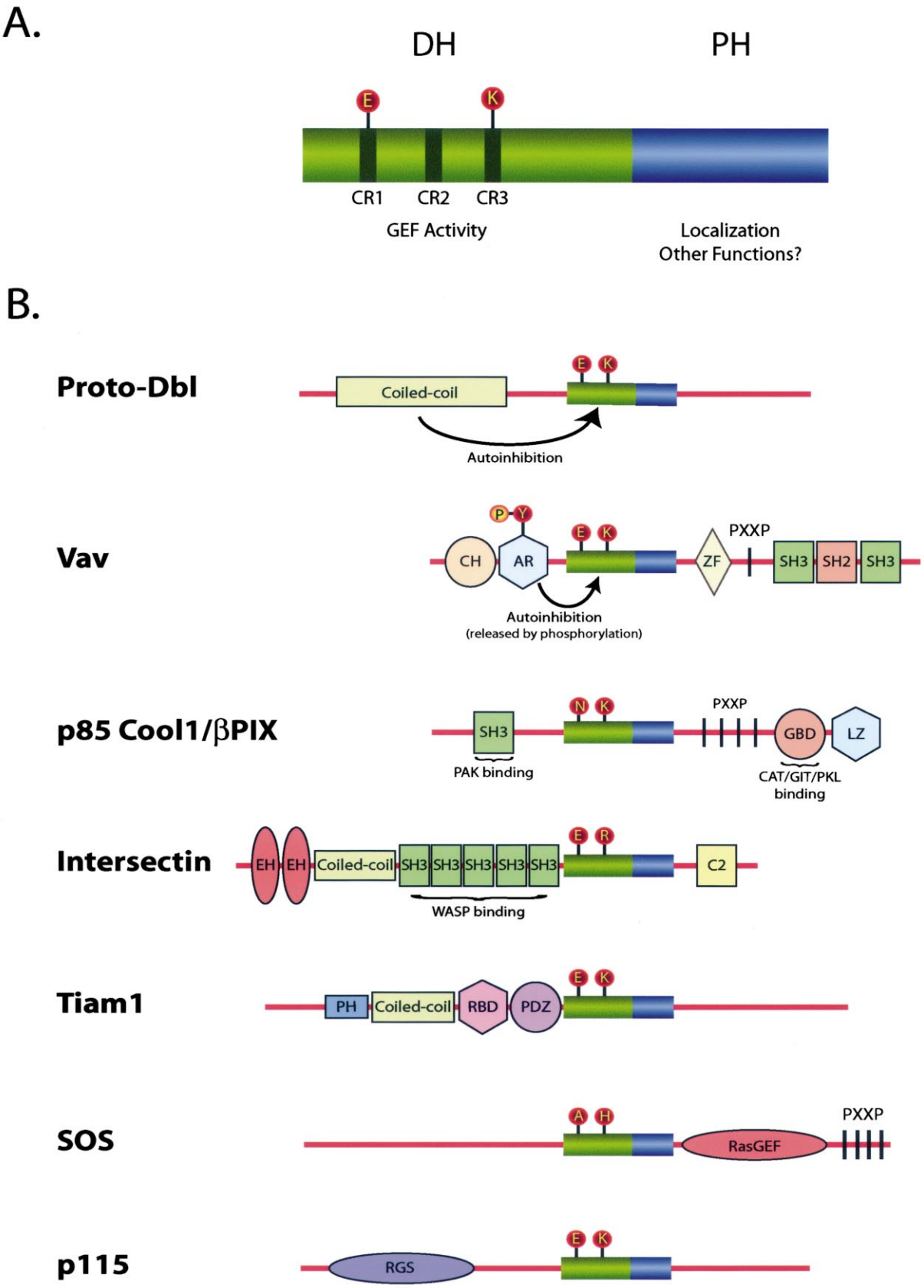

Fig. 1. Diagram of DH domain-containing proteins. A: A schematic diagram of the DH–PH module is shown with the three conserved regions (CRs) of primary sequence homology within the DH domain indicated as dark green boxes. The positions of the conserved glutamic acid in CR1 and lysine in CR3, shown to be critical to the exchange mechanism in the Rac/Tiam1 complex, are indicated (E1047 and K1195 respectively in Tiam1). B: The domain architecture of a number of Dbl family proteins discussed in the text are shown. The DH–PH module is shown as in A, with the residues at the corresponding positions equivalent to E1047 and K1195 in Tiam1 indicated.

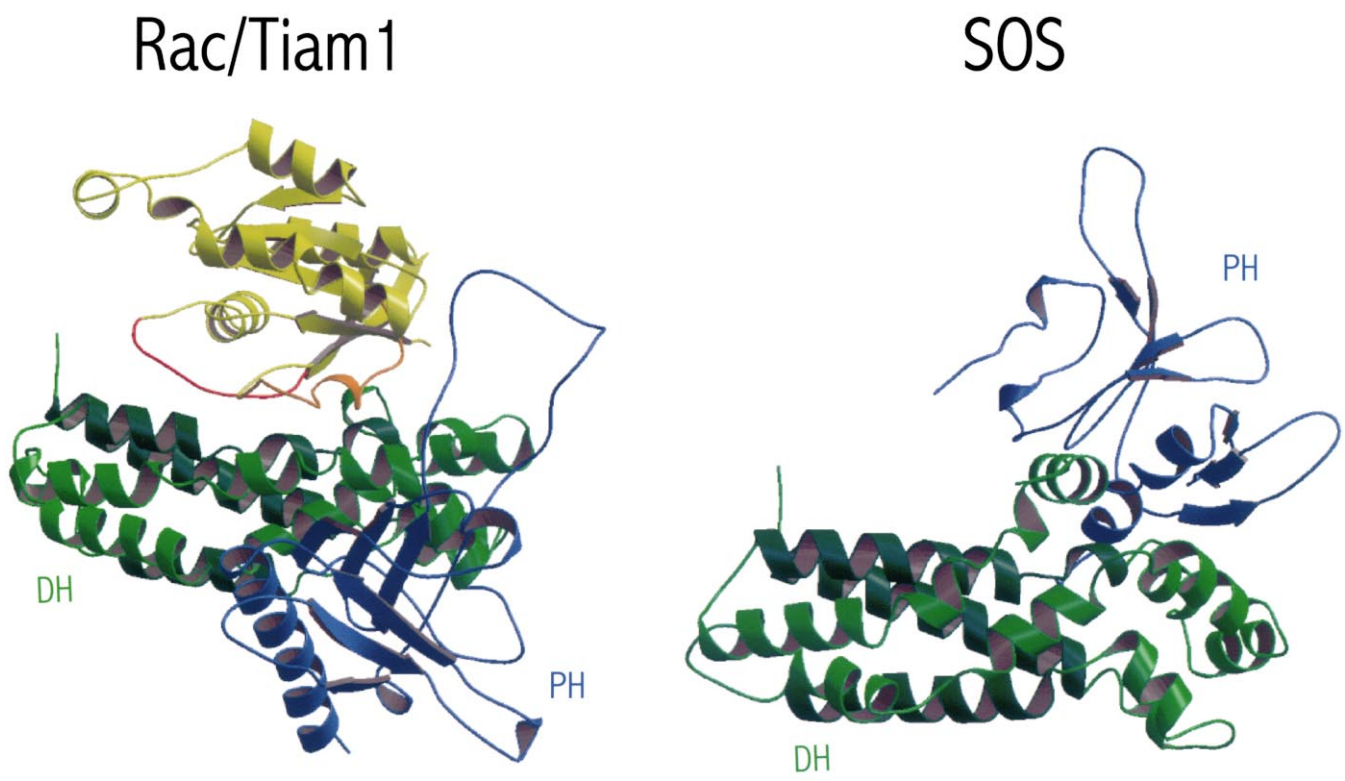

Fig. 2. The structure of Rac in complex with the DH–PH domain of Tiam1 is compared to the structure of the SOS DH–PH domain. The DH domains are green, with the conserved regions (CR1–3) indicated by a darker shade of green. The PH domains are blue and the switch I and switch II regions of Rac are shaded red and orange respectively. The two structures were positioned by least squares superposition of their DH domains to illustrate that the PH domain of SOS is dramatically reoriented relative to the PH domain of Tiam1 and partially occludes the GTPase-binding site.

protrusion formed by the α7-helix immediately adjacent to the conserved surface. This region of the DH domain contacts non-conserved residues in Rac between the β2–3-strands. The corresponding residues in Rho and Cdc42 would not support these interactions and the $\alpha7_{DH}/\beta2–3_{GTPase}$ interaction is likely to be responsible for the highly selective nature of the Rac/Tiam1 interaction. Recent biochemical studies confirm these structural predictions, demonstrating that a single point mutant in the β2–β3 region effectively reverses the specificity of the GTPase/GEF interaction, with the W58F mutation of Rac conferring sensitivity to the Cdc42-specific GEF intersectin and blocking exchange by Tiam1, while the corresponding mutation in Cdc42 (F58W) confers sensitivity to Tiam1 and blocks exchange by intersectin [22]. DH domains have adopted a strategy in which non-conserved residues are responsible for mediating specific binding with the substrate GTPase through regions outside of the switch domains. Following the initial binding event, conserved residues responsible for the exchange activity of the DH domain engage the switch regions to eject the bound nucleotide. Additional structures of DH domains with differing specificities in complex with their Rho family substrates should provide further insights into how specificity is manifested within this diverse family of GEFs.

5. PH domain structure

The structure of the DH–PH module of SOS and the DH–PH domain of Tiam1 bound to Rac are at present the only two structures for Dbl family members that include both the DH and PH domains. As appreciated from earlier NMR structures of the SOS PH domain [23,24], it is now clear that PH domains of Dbl family proteins diverge from the canonical PH structure in their amino-terminal region. In primary sequence alignments of Dbl family proteins, this region is typically included as part of the DH domain, however, structurally it is actually incorporated into the PH domain. In SOS, the PH domain is connected to the DH domain by a flexible linker, and the unique amino-terminal region folds into an α-helix (αN) followed by a short β-strand and a 3_{10}-helix. This structure packs against the β1–β4 sheet on the face that is normally solvent exposed in other PH domain structures and opposite the inositide-binding site. A similar structure is seen in the PH domain of Tiam1, although there is no flexible linker between the domains, and the carboxyl-terminal helix of the DH domain (α9 of Tiam1) extends directly into the PH domain forming a structure analogous to αN of SOS.

While the general structural features of the Tiam1 and SOS PH domains are similar, as illustrated in Fig. 2, the orientation of the PH domain relative to the DH domain in these two structures is dramatically different, with important functional implications to their GEF activity. The PH domain of Tiam1 lies about 55 Å from its position in the SOS structure. In both cases, there is a large complementary surface at the DH–PH interface, but the interface is on different surfaces of the DH domain. Disruption of this interface would impose a large energetic penalty, and the position of the PH domain of Tiam1 is not likely to change upon GTPase binding. In addition, changes in the relative orientation of the PH domain of

Tiam1 would require restructuring of the extended α9-helix, making ensemble changes in the position of this PH domain very unlikely. Surprisingly, the position of the PH domain in the SOS structure largely occludes the GTPase-binding site, and significant steric clash with the PH domain would prevent the SOS DH domain from associating with a GTPase in a manner analogous to the Rac/Tiam1 complex. Importantly, the DH–PH domain of SOS, for which the X-ray crystallographic structure was solved, does not exhibit GEF activity in vitro, while the limit DH domain is active toward Rac, consistent with an inhibitory role for the PH domain [25]. In vivo, the DH–PH domain of SOS was shown to be competent for exchange under conditions that lead to activation of phosphoinositide-3 kinase, suggesting a role for lipid regulation of SOS exchange activity. The guanine nucleotide exchange activity of the SOS DH domain would require movement of the PH domain to permit access to the GTPase-binding site. The available in vivo data are consistent with the possibility that such changes are mediated by phosphoinositide binding, but a direct demonstration of such regulation awaits further structural and biochemical analysis, and the question of allosteric regulation of the DH domain by phosphoinositide binding is a matter of considerable debate (see below).

6. Functional role of the PH domain

Given the wide variation in structure between SOS and Tiam1 with regard to the orientation of the DH–PH interface, it is apparent that there is no satisfactory structural explanation for the conserved pairing of domains seen in Dbl family proteins. However some insight can be provided from the available functional data. In vivo studies of the transforming activities of Dbl family proteins have clearly established that the minimal functional unit for transformation is the intact DH–PH module [11]. While the isolated DH domains often retain full nucleotide exchange activity, truncation of the PH domain abolishes the transforming phenotypes typically associated with these oncogenes. Phosphoinositides remain the only well-characterized ligands for PH domains [12], and it appears that the function of the PH domain is to properly localize the DH domain to its cellular site of action through lipid binding. In this regard, the PH domain of Dbl family proteins is similar to the proline-rich domain of certain Ras family GEFs which bind to SH3 modules in adaptor proteins like Grb2 in order to localize the RasGEF domain to the membrane and couple its nucleotide exchange activity to the activation of receptor tyrosine kinases [26].

Allosteric regulation of the DH domain through lipid binding at the PH domain is an attractive explanation for the conserved coupling of the DH and PH modules, but the available experimental evidence remains controversial. In the case of Tiam1, the binding of the same phosphoinositide to the PH domain has been reported by different authors as inhibitory [27], essential [28], or not involved [29] in the ability of the DH domain to exert exchange activity toward Rac. In an important study by Sondek and colleagues [29], the PH domains of Tiam1, intersectin, and Dbs were all shown to interact with phospholipids with varying specificities but, under conditions where lipid binding was carefully documented, no modulation of nucleotide exchange activity was detected. These data clearly demonstrate that allosteric regulation of DH domains by their associated PH domain is not a conserved feature of Dbl family proteins. As noted in this study, the presentation of phosphoinositides in the appropriate context of a lipid vesicle is essential for assessing the role of lipid binding in regulating GEF activity, but also complicates normal filtration assays used for studying exchange activity by ^{3}H-GDP release. Other biophysical techniques, most notably fluorescence spectroscopy, may provide a more reliable method in the future for assessing the role of lipids in the regulation of GEF activity. These studies do not rule out a regulatory role for the PH domain in Dbl-mediated exchange. Indeed, rearrangements of the PH domains of certain Dbl family members, induced by lipid binding, may cause structural changes in the surrounding loop regions that would impact on GEF activity. Further biochemical studies will be necessary to resolve this issue. In addition, most of these reports take advantage of non-prenylated GTPases, and the coordinated membrane localization of the GTPase/GEF complex through prenylation of the Rho protein and the GEF PH domain may impact on the nucleotide exchange activity of the DH domain through unappreciated mechanisms. Clearly, the PH domain is critical for the function of Dbl family proteins, but a mechanism by which the PH domain influences the GEF activity of these proteins has yet to be firmly established.

7. Activation of the DH domain nucleotide exchange activity

One of the major and as yet unanswered questions regarding members of the Dbl family concerns the mechanism by which upstream signaling events regulate their nucleotide exchange activity. Outside of the conserved DH–PH region, the Dbl proteins have varied sequence and are typically characterized by the presence of multiple signal transduction modules (Fig. 1) [4]. These signaling modules are almost certainly involved in regulatory interactions that modulate the nucleotide exchange activity of the DH domain. In a few cases, the mechanism of this regulation has been established. For example, the binding of $G\alpha_{13}$ to the RGS domain of p115 activates its RhoGEF activity presumably by releasing an inhibitory constraint on the DH domain [30]. The Vav oncogene provides another well-characterized example of regulated nucleotide exchange activity. The oncogenic mutation of Vav involves deletion of a phosphorylation site for Src and Syk family kinases [31,32]. In proto-Vav, phosphorylation of Tyr174 leads to activation of its GEF activity. The structure of Vav bound to a portion of its auto-inhibitory domain including this tyrosine was recently solved by NMR spectroscopy, revealing the mechanism of activation [17]. Tyr174 lies at the center of an inhibitory helix and binds in a complementary cleft on the conserved face of the DH domain, sterically blocking access to the GTPase-binding site. The inhibitory helix interacts with a number of conserved residues in the DH domain, most notably the conserved glutamic acid residue of CR1 (E206, equivalent to E1047 in Tiam1) responsible for interacting with switch I and disrupting the Mg^{2+}-binding site of the GTPase. Phosphorylation of this tyrosine would sterically interfere with binding to the DH domain and phosphorylation also appears to destabilize the helical structure of the inhibitory arm further contributing to release of the auto-inhibitory interaction and the resultant up regulation of GEF activity. Importantly, lipid binding to the PH domain influences the phosphorylation of Tyr174 [31] suggesting a cooperative role for the PH domain in the inhibitory mechanism.

A similar auto-regulatory interaction was recently demonstrated between the amino-terminal coiled-coil region of Dbl and the PH domain [33], suggesting that auto-inhibition by additional signaling modules outside the DH–PH domain may be a general feature of signal transduction through Dbl family proteins. Identifying binding partners for these domains and understanding the specific mechanisms that couple upstream signaling events to the activation of the DH domain represent exciting and important areas of research, which ultimately will shed light on how the nucleotide exchange activity of these proteins is regulated in the context of specific signaling events in the cell.

8. Target effectors as direct regulators of GEF activity

The distinct domains that are present in many Dbl family proteins suggest that they act to integrate multiple inputs in complicated signaling networks involving the Rho GTPases. A startling example of the complex signaling mediated by these proteins is the recent finding that certain Dbl family proteins are able to directly associate with the downstream effectors activated by the GTP-bound form of their Rho family substrates. First, the Cool/PIX proteins (for cloned out of library or Pak interactive exchange factor) were isolated as binding partners for the Ser/Thr kinase Pak (for p21-activated kinase), an effector of Cdc42 and Rac [34–36]. Surprisingly, these proteins were found to contain a DH domain that was reported to have GEF activity toward Rac in vivo [35]. More recently, the Cdc42-specific nucleotide exchange factor intersectin, associated with clathrin-mediated endocytic pathways, was found to bind directly to N-WASP [37], a Cdc42 effector responsible for mediating actin polymerization. Binding of the WASP proline-rich domain to an SH3 domain of intersectin stimulates intersectin's ability to promote nucleotide exchange on Cdc42. In vivo data suggest that Cdc42 localizes to the activated WASP/GEF complex and following nucleotide exchange, Cdc42 interacts with WASP in a GTP-dependent manner to stimulate actin polymerization. Regulation of nucleotide exchange activity through the binding of Pak to Cool/PIX has yet to be demonstrated, but the parallels with the WASP/intersectin complex are striking. In addition to binding Pak, Cool/PIX proteins also associate with the CAT/GIT/PKL proteins [38–40], which are involved in a range of functions including Arf signaling and assembly of focal complexes.

9. Future directions

The Dbl family of proteins, characterized by their conserved DH domain, represents a large group of proto-oncogenes involved in cell growth regulation. The traditional role of these molecules as GEFs for Rho family proteins is now understood in molecular detail from multiple structural perspectives. The challenge for the future will be to delineate the determinants responsible for specificity, as well as understanding how the GEF activity of Dbl family members is integrated into the complex signaling networks that control fundamentally important processes in cell biology ranging from regulation of the actin cytoskeletal architecture, to polarity-dependent processes, and the control of cell cycle progression.

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