GEF what? Dock180 and related proteins help Rac to polarize cells in new ways

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Abstract

Rho GTPase activation, which is mediated by guanine nucleotide exchange factors (GEFs), is tightly regulated in time and space. Although Rho GTPases have a significant role in many biological events, they are best known for their ability to restructure the actin cytoskeleton profoundly through the activation of specific downstream effectors. Two distinct families of GEFs for Rho GTPases have been reported so far, based on the features of their catalytic domains: firstly, the classical GEFs, which contain a Dbl homology–pleckstrin homology domain module with GEF activity, and secondly, the Dock180-related GEFs, which contain a Dock homology region-2 domain that catalyzes guanine nucleotide exchange on Rho GTPases. Recent exciting data suggest key roles for the DHR-2 domain-containing GEFs in a wide variety of fundamentally important biological functions, including cell migration, phagocytosis of apoptotic cells, myoblast fusion and neuronal polarization.

Introduction

Approximately 150 Ras-related small GTPases are found in eukaryotic genomes. Among these, the Rho GTPases are a subfamily represented by 22 members that are best known for their roles in regulating the actin cytoskeleton. Following more than a decade of intense research, Rho proteins have now been implicated in a broad spectrum of biological functions, such as cell motility and invasion, cell growth, cell survival, cell polarity, clearance of apoptotic cells and axonal guidance. The basic biochemical principle for the function of the Rho GTPases, similarly to other small GTPases, is simple: they are bimolecular switches which are ‘on’ when bound to GTP and ‘off’ when bound to GDP [1]. The regulation of this cycling between the GDP and GTP bound states is, however, complex. The activation status of the Rho GTPases is regulated by two antagonistic classes of proteins: (i) the guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP, and (ii) the GTPase-activating proteins (GAPs), which enhance the intrinsic GTPase activity of the Rho proteins, shifting their equilibrium toward the GDP-bound state [1]. An additional level of regulation for the Rho GTPases is provided by the

Rho guanine nucleotide-dissociation inhibitors (RhoGDIs), which sequester the Rho GTPases in a GDP-bound state in the cytosol.

GEFs for Rho GTPases can be subdivided into two main subfamilies. First, the classical Dbl homology–pleckstrin homology domain (DH-PH)-containing family is currently represented by 69 members in mammalian genomes [2]. Second, Dock180-related proteins containing the Dock homology region (DHR)-2 domain (also known as Docker–ZH2 domain) form a subfamily of 11 mammalian members [3–5] (Box 1). Recent reviews have addressed the basic properties of the DH-PH-containing GEFs and Dock180-related GEFs [2,3,6]. Here, we focus on the recent advances that have been made in connection with the Dock180, Dock2 and Dock7 molecules. New information on the regulation and spatiotemporal localization of these molecules in cells has recently been obtained, and is likely to provide significant insights into the function and regulation of the Dock180 superfamily of proteins at large. We also discuss the biological role of these proteins in cell migration and axon specification.

Box 1

The signature of the Dock180-related proteins: DHR-1 and DHR-2 domains

In mammals, 11 Dock180-related proteins have been identified, and they have been named Dock1 (also known as Dock180) to Dock11 (Figure I). The family members can be further classified into four subfamilies, which, in turn, have been denoted Dock-A, -B, -C and -D (see later) [14]. Dock180 was originally identified as a c-Crk-binding protein, and the PxxP-domain in the C-terminus of Dock180 that mediates this interaction is conserved among several members of the Dock-A and Dock-B subfamilies.

GEFs of the Dock180 superfamily all share the presence of two evolutionarily conserved protein domains, termed DHR-1 and DHR-2 [14,41]. Similarly to DH-PH modules, the DHR-2 domains of several members of this family have been shown to interact with the nucleotide-free form of the Rho GTPase that they catalytically target [4,5,12,14,42–44]. This interaction of GEF with the nucleotide-free GTPase reflects an intermediate in the catalytic reaction leading to the exchange of GDP for GTP on the GTPase [4,5,12,14,38,42–45]. Accordingly, several DHR-2 domains have been shown to be both necessary and sufficient to promote specific guanine nucleotide exchange on various GTPases, both *in vitro* and *in vivo*. Despite virtually identical biochemical properties between the DHR-2 and DH-PH GEF modules, their primary amino acid sequences are disparate. Inactivation of the DHR-2 domain in Dock180 has been shown to block Rac activation, cell migration and phagocytosis, highlighting the importance of this domain in the biological function of the protein [4,11,14].

The DHR-1 domain is a unique evolutionarily conserved domain invariantly located upstream of the DHR-2 domain in all Dock180-related GEFs [14,41]. Weak homology to the C2 domain, which is a well characterized lipid-binding module, can be detected at the primary amino acid sequence of DHR-1 for a subset of Dock180-related proteins [14]. In the case of Dock180, the DHR-1 domain was recently shown to mediate a specific interaction with phosphatidylinositol (3,5)-bisphosphate and PtdIns(3,4,5) P_3 signaling lipids *in vitro* and in cells [16]. Significantly, mutations in the DHR-1 domain of

Dock180 block Rac-dependent cell elongation and cell migration, despite unaffected Rac GTP loading in these cells. These results highlight the important difference between ‘Rac activation’ (as measured by GTP loading) and ‘Rac signaling’ (as measured by biological output). Thus, the DHR-1 domain seems to have a fundamental role in Dock180–Rac signaling by positioning and promoting Rac activation at the sites of PtdIns(3,4,5) P_3 production by PtdIns 3-kinase (Figure 5), thereby leading to productive Rac signaling. Figure I.

Figure 1.

Dock180 and Elmo: a productive union in Rac signaling

Dock180 was originally identified as a binding protein for the SH3 domain of the proto-oncogene product c-Crk through its C-terminal PxxP region [7] (Box 1). Later on, biochemical studies in mammalian cells demonstrated that Dock180 was positioned upstream of the Rho family member Rac [8]. Subsequent genetic screens in *Caenorhabditis elegans* and *Drosophila melanogaster* suggested that the Dock180 orthologs in these organisms were also functioning upstream of Rac in a range of biological events, including phagocytosis of apoptotic cells, migration of gonad cells and myoblast fusion [9,10]. Studies in *C. elegans* have especially paved the way to a more detailed understanding of the regulation of the Dock180 signaling pathway.

Ced-12 (ortholog of mammalian Elmo; ‘Ced’ standing for ‘cell death abnormal’) was identified as a component of a genetic signaling cascade in *C. elegans* that also contains Ced-2 (ortholog of mammalian c-Crk) and Ced-5 (ortholog of mammalian Dock180). This signaling cascade controls the activity of Ced-10 (ortholog of mammalian Rac) during cell migration and phagocytosis of apoptotic cells in *C. elegans* (Box 2). Ced-12 is an evolutionarily conserved protein, and three family members, Elmo1, -2 and -3, have been identified in mammals.

The Elmo proteins seem to be scaffold proteins, with no obvious catalytic activity. They share conserved domain features, including armadillo repeats at the N-terminus, an atypical PH domain, and a complex proline-rich region at the extreme C-terminus (Figure 1). As depicted in Figure 1, Elmo1 and Elmo2 proteins have been shown to interact physically with four mammalian Dock180 proteins that contain an SH3 domain, namely Dock180 (also known as Dock1), Dock2, Dock3 and Dock4 [11,12] (Dock5 also contains an SH3 domain but its interaction with the Elmo proteins has not been experimentally demonstrated to date). The mechanism for interaction between Elmo and Dock180 seems to be complex and remains to be fully elucidated. Although the SH3 domain of Dock180 binds to the proline-rich region in Elmo, this binding is dispensable in coimmunoprecipitation and pull-down experiments [13]. Clearly, the crucial domains required for the Elmo–Dock180 interaction remain to be mapped [4]. What are the consequences of the Elmo–Dock180 interaction?

Two independent (but not mutually exclusive) roles for Elmo in Dock180–Rac signaling that are now being uncovered are discussed here.

Box 2

Overview of the roles of Ced-5 and MBC, the orthologs of mammalian Dock180, in worms and flies, respectively

Genetic screens in *C. elegans* were central to the identification of genes and gene products involved in the regulation of programmed cell death, or apoptosis (Figure I). Pioneering work led to the discovery of several *Ced* genes and their positioning in genetic signaling cascades. Among these, two distinct sets of genes were shown to regulate the engulfment of dying cells, which is the last step in apoptosis, during embryogenesis: *Ced-1*, *Ced-6* and *Ced-7* genes [orthologs of mammalian MEGF-10 (multiple EGF-like domains-10)] Gulp and ABC transporter, respectively) form one signaling pathway, and *Ced-2*, *Ced-12*, *Ced-5* and *Ced-10* genes (orthologs of mammalian c-Crk, Elmo, Dock180 and Rac, respectively) form the other [46]. Because Ced-5 and Ced-12 bind directly to a PtdSer receptor in phagocytes, the Ced-5–Ced-12 complex has been proposed to integrate the activation of the Ced-2–Ced-5–Ced-12–Ced-10 signaling cascade during the early stage of recognition of apoptotic cells, exposing the ‘eat-me’ signal PtdSer [23] (Figure 3). Interestingly, mutations in the *Ced-2*, *Ced-5*, *Ced-12* and *Ced-10* genes result not only in defects in the engulfment process, but also in a failure of distal tip cells of the gonads to migrate correctly [26], and also in defects in the outgrowth of D-type motor neurons and in the migration of P-cells during brain development [47]. Presently, the molecular mechanisms downstream of Ced-10 that lead to cell motility and phagocytosis remain to be elucidated.

MBC, the *Drosophila* ortholog of Dock180, was discovered in a screen for genes controlling the fusion of myoblasts into multi-nucleate muscle fibers [9,34]. MBC was proposed to function directly upstream of dRac (*Drosophila* Rac protein) during myoblast fusion [48]. Interestingly, MBC mutant myoblasts can still align with one another and make initial cell–cell contact, but they are unable to remodel their membranes for fusion. MBC is also involved in, but is not absolutely required for, at least three different types of cell migration events in *Drosophila*. First, the migration of border cells toward the oocyte, in which the migratory attractant Platelet-derived growth factor-vascular endothelial growth factor (PVF) is highly expressed, is regulated by the platelet-derived growth factor–vascular endothelial growth factor receptor (PVR) and the Rac GTPase. Clonal analyses revealed that among several candidate genes that regulate Rac activation, only MBC mutations significantly delayed the migration of border cells [49]. Second, halfway through embryogenesis, MBC is expressed in the epidermis and seems to have an important role in the movement of the ventral and lateral epidermis to surround the embryo. This process, termed ‘dorsal closure’, involves a collective movement of epithelial cells, whereby the leading edge cells guide the movement of the sheets. In MBC mutant embryos, similarly to Rac mutants, F-actin is significantly less abundant in the leading edge cells, suggesting that the MBC–Rac signaling pathway might directly regulate the actin cytoskeleton; however, this remains to be demonstrated [34]. Third, during metamorphosis, in a process termed ‘thorax closure’, the dorsal regions of the

wing imaginal discs migrate towards each other, eventually to fuse at the midline to form the notum. This process is similar to dorsal closure, and many of the same genes are required for both events. A kinase cascade through MBC, Rac and c-Jun N-terminal kinase (Jnk) seems to be essential, downstream of the PVR, for thorax closure to occur normally [50].

Figure 1.

The Dock180–Elmo complex and the bipartite GEF model

Some studies suggest that Dock180 is catalytically active toward Rac only when in complex with Elmo [4]. In support of this hypothesis, deletion mutants of Dock180 that fail to bind to Elmo do not significantly activate Rac when over-expressed in cells [11]. One possible explanation put forth for this ‘synergy model’ is that the binding of Elmo to Dock180 increases the affinity of this protein complex towards nucleotide-free Rac, thus favoring the formation of a key intermediate during the catalysis of GDP for GTP exchange [4]. Mechanistically, the atypical PH domain of Elmo was shown to be the key determinant in increasing the catalytic activity of Dock180 towards Rac. The PH domain of Elmo does not interact directly with either Dock180 or Rac. Instead, it can bind ‘*in trans*’ to the Dock180–nucleotide-free Rac complex to stabilize a trimolecular complex between the three proteins [13] (Figure 2). This action of the Elmo PH domain was shown modestly to increase the catalytic activity of Dock180 toward Rac, by about twofold *in vitro*. Nevertheless, it was found that this effect could be significant *in vivo* because the expression of a form of Ced-12 with mutations in the PH domain failed to rescue the migration defects in Ced-12-null worms [13]. However, many groups have demonstrated that overexpression of Dock180 alone is sufficient to activate Rac, and that an isolated DHR-2 domain of Dock180 (in the absence of Elmo) efficiently exchanges on Rac both *in vitro* and in mammalian cells [14] (Box 1). Clearly, further studies are needed to reveal the exact contribution of Elmo in the Dock180-mediated activation of Rac. Elmo is clearly crucial for efficient Rac signaling by Dock180 during cell migration and phagocytosis. This could, in part, also be due to the ability of Elmo to localize the complex in defined cellular compartments, which is

instrumental for the subsequent activation of biological signaling events downstream of Rac. This possibility is discussed later.

Elmo might aid targeting of Dock180 to the plasma membrane

Four different mechanisms for recruiting the Elmo–Dock180 complex to specific binding partners at the plasma membrane have recently been uncovered (Figure 3). These partners include RhoG, Arf6, the phosphatidylserine (PtdSer) receptor and the IpgB-1 protein from *Shigella*. The potential importance of these interactions is now discussed.

RhoG—Recent studies have demonstrated the N-terminal portion of Elmo to be a binding partner for the constitutively active RhoG GTPase [15]. It was shown that the recruitment of the Elmo–Dock180 complex to the active RhoG molecule, which is mainly located at the plasma membrane, is crucial for efficient Rac-dependent epithelial cell spreading on the matrix protein fibronectin [15]. Thus, abrogation of the Elmo–Dock180 complex by dominant-negative proteins or by small interfering RNAs (siRNAs) against Elmo, RhoG or Dock180 significantly delayed cell spreading and reduced cell migration on fibronectin [15–17]. In addition, in PC 12 rat pheochromocytoma cells, interfering with the RhoG–Elmo pathway blocked Rac-dependent neurite outgrowth induced by both neural growth factor and serum. Interestingly, RhoG has also been shown to mediate the recruitment of the Elmo–Dock180 complex to the plasma membrane during engulfment of latex beads that mimic apoptotic cells [18]. In *C. elegans*, UNC-73 (mammalian Trio), which is a GEF for Mig-2 (mammalian RhoG), functions as the direct upstream activator enabling coupling between Mig-2 and Ced-12, triggering the Ced-5-mediated activation of Ced-10 at the membrane during engulfment [18]. These genetic results are not completely understood because a double mutation in the UNC-73 and Mig-2 genes does not induce engulfment defects. Nevertheless, the *UNC-73 Mig-2* double mutant enhances the defects observed in *Ced-2*, *Ced-5*, *Ced-12* and *Ced-10* gene mutants. The biological importance of this signaling cascade in mammalian cells remains to be fully determined because deletion of the *RhoG* gene in mice does not result in any obvious phenotype [19].

Arf6—Similarly to RhoG, Arf6, another GTPase known to activate Rac, could also mediate the recruitment of the Elmo–Dock180 complex to the plasma membrane [20]. Thus, Arno, which is a specific GEF for Arf6, has been shown to promote strong targeting of Elmo–Dock180 to the membrane, and dominant-negative forms of both Dock180 and Elmo block Arno-induced lamellipodia formation and cell migration in a Madin–Darby canine kidney cell model [20]. It is not clear whether a direct interaction between Arf6 and Elmo (or Dock180) exists. Interestingly, in *Drosophila*, Arf6 and its GEF, Loner, which is an Arno-related Sec7 domain-containing GEF, were shown to regulate Rac localization and activation during myoblast fusion [21]. It will be interesting to investigate if Arf6 and Loner do so by recruiting the dElmo–Myoblast City (MBC; *Drosophila* Dock180) complex to promote dRac activation during myoblast fusion.

PtdSer receptor—During apoptosis, dying cells expose the lipid PtdSer on the outer side of the plasma membrane as an ‘eat-me’ signal [22]. Phagocytes subsequently recognize this signal to engulf the apoptotic cells through a PtdSer receptor. Identification of the receptor

for PtdSer in mammals preceded its discovery in *C. elegans*. However, genetic studies in worms placed the PtdSer receptor in the same pathway as Ced-2, Ced-5, Ced-10 and Ced-12 during engulfment of cell corpses [23]. Mechanistically, both Ced-5 and Ced-12 have been shown to interact directly with the PtdSer receptor in *in vitro* binding assays [23]. This suggests that the recruitment of the Ced-10-activating machinery at the membrane can be accomplished through specific interactions with the PtdSer receptor. It remains to be determined whether the mammalian PtdSer receptor(s) also directly recruit Elmo–Dock180 following ligand binding. It also raises the question of the role of Ced-2 in this pathway because this molecule was expected to have such a receptor-targeting role. Further studies are thus needed to understand the precise roles of these genes in the engulfment of apoptotic cells.

IpgB-1—It has been shown that the evolutionarily conserved pathways for phagocytosis described earlier are used by some pathogens to infect mammalian cells. Thus, phagocytic-like events regulate the ability of certain types of bacteria, such as *Shigella* and *Salmonella*, to enter intestinal epithelial cells. To promote membrane protrusions, *Shigella* injects proteins into epithelial cells, and one of these bacterial proteins, IpgB1, was recently shown to bind directly to Elmo and recruit the Elmo–Dock180 complex to the membrane [24]. Interfering with IpgB1–Elmo complex formation significantly decreased the ability of *Shigella* to infect epithelial cells in culture [24]. Inhibition of the Elmo–Dock180 pathway could thus provide a novel means to prevent infection by this pathogen.

Old tricks for new GEFs: how to regulate Dock180 function

Common themes on how signaling proteins are regulated include regulation by autoinhibitory domains, by post-translational modifications such as phosphorylation, and through targeted degradation of the signaling molecule. Not surprisingly, Dock180 has been shown to be subject to such regulation. Some of these recently uncovered regulatory mechanisms, the functions of which are likely to prevent aberrant Rac activation, are summarized below and in Figure 4.

Autoregulation of Dock180 GEF activity by its SH3 domain

Overexpression of a form of Dock180 that lacks its SH3 domain results in 1.5-fold higher Rac GTP-loading compared with Rac activation observed following overexpression of a wild-type form of Dock180. It was therefore suggested that the SH3 domain of Dock180 might have autoinhibitory properties [25]. In support of this, it has been shown that the SH3 domain can bind directly to the DHR-2 domain of Dock180. Interestingly, this interaction is not dependent on a proline-rich region within the DHR-2 domain. This binding event has been suggested to lead to the inhibition of the ability of the DHR-2 domain to bind to nucleotide-free Rac and to promote its GTP loading [25]. Consequently, one could envision that Elmo might promote Dock180 GEF activity toward Rac by binding to the SH3 domain of Dock180 (see earlier), thus exposing the DHR-2 domain to bind to and activate Rac. At the present time, it is unclear whether, in cells, such regulation takes place in a stimulus-dependent manner. Thus far, available data have demonstrated, rather, that the binding

between Dock180 and Elmo seems to be close to stoichiometric and constitutive in cells [16,26].

Ubiquitylation of Dock180

Based on the observations that overexpressed Dock180 protein is more stable when it is co-overexpressed with Elmo, and that endogenous Dock180 becomes more rapidly degraded when Elmo is knocked down by siRNA, it was hypothesized that one role of Elmo could be to stabilize Dock180. As a corollary, it was speculated that the proteasome might be involved in degrading the 'Elmo-free' Dock180 protein [27]. Interestingly, these results might explain, in part, why Dock180 seems to be more catalytically active towards Rac when coexpressed with Elmo –that is, because of an increased stability of the Dock180 protein in the presence of Elmo, rather than because of an increase in its catalytic activity *per se* [27]. Dock180 is indeed ubiquitylated in cells, and this can be partially blocked by coexpressing Elmo or portions of Elmo that interact with Dock180. Interestingly, overexpression of c-Crk had the opposite effect and led to an increase in Dock180 ubiquitylation. It will be essential to investigate if endogenous Dock180 is also a target for ubiquitylation, and, if so, what is the biological relevance of the potential ubiquitin-mediated degradation of this GEF, and the role of Elmo in this regulation.

Phosphorylation of Dock180 and Elmo

Soon after its discovery, Dock180 was shown to become serine/threonine phosphorylated specifically following integrin engagement to extracellular matrix [28]. The functional consequence of this phosphorylation remains poorly understood. Similarly, Elmo is phosphorylated on tyrosine residues when the Src family kinase Hck is overexpressed; this seems to be important for Elmo- and Dock180-induced migration and phagocytosis in overexpression models [29,30]. However, because there is no evidence that endogenous Elmo is tyrosine phosphorylated during either engulfment or cell migration, the relevance of these findings remains undefined.

Dock180 proteins in cell polarization and fusion: localize me to PtdIns(3,4,5) P_3 !

Many biological events, such as cell migration, phagocytosis, asymmetric cell division and axon specification in neurons, require cellular polarity. For example, to move in a directional manner, cells polarize towards the migratory attractant and form a filamentous actin-rich leading edge. In addition to the asymmetry in cell shape, signaling molecules controlling the generation of this actin-rich structure and the traction force required for cell movement also become concentrated at the leading edge. Protein complexes that are central regulators of cell polarity have been identified [31]. Additionally, the lipid second messenger phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5) P_3] seems to be key for the appropriate localization of these signaling molecules required for cell polarization, and for the initial establishment of the leading edge [32]. Importantly, Dock180 was shown to bind to PtdIns(3,4,5) P_3 [33]. Other Dock180-related proteins, namely Dock2, Dock7 and MBC, have also been reported to bind to PtdIns(3,4,5) P_3 , and the relevance of these interactions is discussed below and in Figure 5.

Dock180 and PtdIns(3,4,5)P₃

The DHR-1 domain has previously been identified as an evolutionarily conserved domain in Dock180-related proteins [14]. Although the function of this domain was unknown at the onset, it was observed that the DHR-1 domain was essential for Dock180-mediated cell elongation. Interestingly, a form of Dock180 that lacks the DHR-1 domain was unable to promote migration, yet it activated Rac, as measured by GTP loading, just as well as its wild-type counterpart [16]. In a search for a molecular function for the DHR-1 domain, some similarity at the level of the primary amino acid sequence was detected between the DHR-1 domain and the C2 domain, which is a versatile lipid-binding module. It was subsequently demonstrated that the DHR-1 domain of Dock180 can specifically interact with PtdIns(3,4,5)P₃, both *in vitro* and *in vivo* [16]. Supporting a role for this interaction, the ability of Dock180 to promote migration was blocked by pharmacological inhibitors against phosphatidylinositol 3-kinase (PI 3-kinase). Furthermore, replacing the DHR-1 domain in a chimeric Dock180 construct with a canonical PtdIns(3,4,5)P₃-binding PH domain resulted in a fully active Dock180 molecule that promoted cell elongation and cell migration. These findings suggest that the role of the DHR-1 domain is to localize Dock180 at membrane sites that are rich in PtdIns(3,4,5)P₃, where Dock180 subsequently activates Rac through its DHR-2 domain. Thus, the cellular asymmetry generated by the PtdIns(3,4,5)P₃ lipid gradient in response to a migratory attractant is essential for directed cell migration (Figure 5). It will be interesting to investigate if the PH domain of Elmo is also involved in lipid interaction, and if it can function in synergy with the DHR-1 domain to localize the Elmo–Dock180 complex at the sites of PtdIns(3,4,5)P₃ production.

MBC, myoblast fusion and PtdIns(3,4,5)P₃

Mutations in the MBC locus lead to severe myoblast fusion defects in *Drosophila* [9,34]. Recently, a structure–function study of the MBC protein was performed, addressing its role in myoblast fusion *in vivo*. It was found that the SH3, DHR-1 and DHR-2 domains of MBC are all essential for correcting the muscle defect in an *mbc* mutant background in rescue experiments. Surprisingly, the interaction between dCrk and MBC was completely dispensable for the rescue of the myoblast fusion phenotype. These authors further found that the DHR-1 domain of MBC binds to PtdIns(3,4,5)P₃ *in vitro*. Although few studies highlight a role for PtdIns lipids in the differentiation of myoblasts in mammalian systems, the requirement of PtdIns(3,4,5)P₃ in MBC signaling at the fusion step reveals an exciting new area of research in myogenesis (Box 2).

Dock2, polarity and PtdIns(3,4,5)P₃

Dock2 is a Dock180-related protein that is primarily expressed in cells of hematological origin (Box 3). Dock2^{−/−} neutrophils fail to activate Rac and to accumulate F-actin at the uropod in response to the chemoattractant N-formyl-methionyl-leucyl-phenylalanine (fMLP) [35]. Furthermore, endogenously expressed Dock2, fused to green fluorescent protein GFP by a knock-in strategy, translocates to the uropod in a PI 3-kinase-dependent manner. Similarly to Dock180, the DHR-1 domain of Dock2 was shown to bind to PtdIns(3,4,5)P₃. Interestingly, it was also noted that Dock2-null neutrophils accumulated less PtdIns(3,4,5)P₃ in response to fMLP, suggesting a role for Dock2 either in the stabilization of the lipid

product or in the activation of PI 3-kinase (Figure 5). It will be interesting to investigate if Dock2, or Dock2-activated Rac, is responsible for the establishment of the positive feedback loop that is known to activate PI 3-kinase during chemosensing in neutrophils [36]. Finally, Dock2 was shown to mediate lymphocyte migration in a PI 3-kinase-independent manner [37], suggesting that the requirement for $\text{PtdIns}(3,4,5)\text{P}_3$ in Dock2 signaling could be cell-type specific.

Box 3

Dock2: a key regulator of immunity

Expression of Dock2 is restricted to hematopoietic cells, and the function of this gene in this cellular compartment has been studied extensively by gene inactivation in mice. Using this tool, it was found that Dock2^{-/-} T and B lymphocytes failed to migrate *in vitro* toward cytokines, and *in vivo*, these cells failed to home in to their natural niche, namely the lymph nodes and the spleen [51]. As a result of the defect in lymphocyte migration, mice lacking Dock2 displayed abnormalities such as atrophy of lymphoid follicles and loss of marginal zone B cells [51]. Furthermore, Dock2 seems to be required in T cell precursors for their development into V α 12 natural killer cells [52]. In addition to their role in immune regulation, these cells have an important role in killing cancer cells, and it will be interesting to investigate the role of Dock2 in this function. These mice also provided a valuable model to investigate the potential therapeutic significance of pharmacological inhibition of Dock2. In this respect, some recent studies suggest that pharmacological inhibition of Dock2 could be beneficial in preventing graft rejection [53].

Dock7, axon specification and $\text{PtdIns}(3,4,5)\text{P}_3$

Dock7 was recently identified as an upstream regulator of Rac in a yeast two-hybrid screen. Additional studies uncovered that Dock7, indeed, is able directly to activate Rac, but not Cdc42 or RhoA, through its DHR-2 domain [38]. Dock7 is highly expressed in the developing rat brain and in hippocampal neurons at stage 2 of development, it was found to be highly abundant in the neurite that subsequently gives rise to the axon. This is somewhat counterintuitive because this is the neurite that shows less actin polymerization. At stage 3, Dock7 was mostly located in the shaft of the axon, where it strongly colocalized with microtubules. This asymmetrical distribution of Dock7 suggested that it might have a role in axon specification. An interesting aspect of this work is that the Dock7–Rac pathway seems to promote axon growth in an actin-independent manner. Mechanistically, Rac seems to activate an undefined kinase that phosphorylates Stathmin (also called Op18), which is a microtubule binding and destabilizing protein, on serine 16. This phosphorylation event inhibits the destabilizing activity of Stathmin, thereby promoting microtubule growth and generation of the axon. How does Dock7 become distributed asymmetrically in neurons? It is known that $\text{PtdIns}(3,4,5)\text{P}_3$ is produced in the forming axon, and Watabe-Uchida *et al.* [38] were able to show that the ability of Dock7 to induce ectopic axons is inhibited by PI 3-kinase inhibitors. It would therefore be of interest to see whether the DHR-1 domain of Dock7 has a role in the asymmetrical distribution of Dock7 to the future site of the axon in

developing neurons (Figure 5). Interestingly, *stathmin* was recently identified as a gene regulating border cell migration during *Drosophila* oogenesis [39]. It will be interesting to study whether Stathmin is regulated by Rac in this invasive process.

Dock180 downstream of the Fak and v-src kinases: invade!

Focal adhesion kinase (Fak) is a central player, integrating both integrin and growth factor signals into cell motility. It was recently demonstrated that the oncogenic form of Src, v-src, can rescue the migration defects observed in Fak-null cells [40]. Surprisingly, v-src was unable to promote invasion in Fak-null cells, and this activity of v-src could only be restored following re-expression of Fak. This suggests an important role for Fak in connecting v-src to the invasion machinery. What could be the target(s) of the Fak–v-src complex for invasion? There is a dramatic increase in p130Cas tyrosine phosphorylation and in the formation of the p130Cas–c-Crk complex in v-src-transformed cells. In Fak-null cells expressing both v-src and Fak, but not in Fak-null cells expressing only v-src, the formation of a multiprotein complex consisting of Fak, v-src, p130Cas, Crk and Dock180 was facilitated. This enhanced protein complex formation correlated with a dramatic increase in Rac-GTP levels and in the extension of invadopodia. These results suggest that Dock180 and its binding partners could have a key role in invasion of transformed cells. These findings warrant further studies investigating the extent to which the Dock180 pathway participates in tumor progression and in metastasis.

Concluding remarks and perspectives

Here, we have briefly overviewed some new and emerging paradigms in the regulation of Dock180 and related molecules, and also mentioned some of the recently elucidated important roles for these proteins *in vivo* in normal and pathological conditions. The Dock180 proteins remain poorly characterized, and future work will reveal the GTPase targets of all of the family members. Elmo proteins are emerging as key regulators of Dock1 (Dock180)-5 proteins, and additional biochemical, genetic and structural studies are required to appreciate fully the role of the Elmo–Dock180 complex in Rac activation. Also unappreciated is how Rac is localized to the membrane following its activation by Dock180. Do Dock180 and Elmo physically bring Rac along? Is Rac distributed in a polarized manner following activation of the Dock180–Elmo pathway, and, if so, is this characteristic only for the GTP-loaded form? Clearly, this will be an exciting area of investigation. Interestingly, members of the Dock-C and Dock-D subfamilies, consisting of Dock6–11, do not bind to Elmo. It will be important to identify regulators of these proteins, to gain insight into their physiological functions in full organisms. In the case of Dock180 itself, and in its *Drosophila* ortholog MBC and *C. elegans* ortholog Ced-5, much is already known on how these proteins activate Rac but the signaling cascades downstream of this GTPase remain largely unidentified. It will also be essential to study the biological functions of the various Dock180 proteins in mouse models to uncover their unique biological functions and their potential involvement in human diseases.

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Figure 1.

Schematic representation of the Elmo proteins and their interaction with the Dock180-related proteins. A large portion of the C-terminal domain of Elmo, including part of the PH domain and the proline-rich region (black bar), is required for Elmo to interact with the Dock180-related proteins (double-headed arrow). The N-terminal portion of Dock180, in turn, is required for Dock180 to bind to the Elmo proteins. Although the SH3 domain of Dock180 can bind to Elmo *in vitro*, some studies suggest that this domain is dispensable for the Dock180–Elmo interaction in cells.

Figure 2.

The bipartite GEF model. In this model, the GEF activity of Dock180 toward the Rac GTPase becomes detectable, or is greatly enhanced, when Dock180 is bound to Elmo. Mechanistically, it is thought that the PH domain of Elmo interacts *in trans* with the nucleotide-free (nf) form of Rac bound to the DHR-2 domain of Dock180. This interaction then increases the affinity of Dock180 toward nf-Rac and the subsequent GTP loading of Rac by the DHR-2 domain of Dock180.

Figure 3.

Mechanisms to target Dock180 to the membrane through Elmo. **(a)** Elmo contains a RhoG-binding domain (in yellow), which specifically recognizes the GTP-bound form of the RhoG GTPase. Following cell adhesion to fibronectin, GTP-bound RhoG recruits the Elmo–Dock180 complex to the plasma membrane, promoting the formation of Rac GTP at these sites. Trio is a GEF that can activate RhoG. **(b)** Following wounding of an epithelial cell monolayer, the Elmo–Dock180 complex localizes and activates the Rac GTPase at the leading edge of the cells migrating to close the wound. In this model, the recruitment of Elmo–Dock180 to the membrane is thought to be mediated by the Arf6 GTPase. Arno is a GEF that can activate Arf6. The exact mechanism by which Arf6 recruits Elmo and/or Dock180 to the membrane remains to be determined. This recruitment could be direct or indirect, through an unidentified bridging protein (depicted as ‘X’). **(c)** In the *C. elegans* model, PtdSer, which is exposed on apoptotic cells, binds to the PtdSer receptor on the surface of phagocytes. This interaction leads to a recruitment of Ced-5 (Dock180), Ced-12 (Elmo) and Ced-2 (Crk) to the receptor in the phagocyte and to subsequent activation of Ced-10 (Rac) and engulfment of the apoptotic cell. **(d)** *Shigella* injects bacterial proteins into the epithelial cells, to activate Rac-mediated membrane ruffling and subsequent entry of the pathogen to the cells. A key bacterial protein for this function, IpgB1, binds directly to Elmo. The Elmo–Dock180 complex then localizes to the membrane and activates Rac at the sites of *Shigella* entry. The binding of Elmo to IpgB1 is mediated by the N-terminal Arm repeats in Elmo, which also contain the RhoG binding site [see (a)].

Figure 4.

Regulation of Dock180 function. **(a)** Autoinhibition of Dock180 by intramolecular interactions. The SH3 domain interacts *in cis* with the DHR-2 domain in the Dock180 molecule, thereby preventing the binding of Rac to the DHR-2 domain. Following Elmo binding to Dock180, the SH3 domain becomes disengaged from the DHR-2 domain, facilitating Rac interaction and subsequent GEF catalysis by the DHR-2 domain. **(b)** Elmo stabilizes the Dock180 protein in cells. In overexpression models, Dock180 becomes ubiquitinated by hitherto unidentified ubiquitin ligases following its dissociation from Elmo, followed by proteasome-mediated degradation of Dock180. Notably, stimuli that promote Elmo–Dock180 dissociation have not been reported. Abbreviation: Ub, ubiquitin. **(c)** Regulation of the activity of the Dock180–Elmo complex by phosphorylation. **(i)** Elmo interacts with the Src family kinase Hck and becomes phosphorylated on specific tyrosine residues. These phosphorylation events increase the GEF activity of the Elmo–Dock180 complex toward the Rac GTPase by an unknown mechanism. Cellular stimuli that lead to

Elmo phosphorylation have not yet been reported. (ii) Integrin engagement on fibroblasts leads to phosphorylation of Dock180 on serine and threonine (S/T) residues. The exact sites of phosphorylation and the consequences of these post-translational modifications on Dock180 are currently unknown.

Figure 5.

Regulation of Dock180-related protein signaling by $\text{PtdIns}(3,4,5)\text{P}_3$. **(a)** The DHR-1 and DHR-2 domains of Dock180 and Dock2 integrate $\text{PtdIns}(3,4,5)\text{P}_3$ lipids with Rac activation at the leading edge. The DHR-1 domain interacts directly with $\text{PtdIns}(3,4,5)\text{P}_3$, and recruits Dock180 and Dock2 to the membrane to activate Rac through their DHR-2 domains. This localized activation of Rac leads to the generation of an actin-rich leading edge, which, in turn, promotes directed movement of the cell toward a migratory attractant (chemokine, cytokine, extracellular matrix gradient). **(b)** MBC promotes myoblast fusion in a $\text{PtdIns}(3,4,5)\text{P}_3$ -dependent manner. (i) Impaired myoblast fusion in *mbc*-null flies. (ii) Transgenic expression of MBC in *mbc*-null flies rescues myoblast fusion defects. (iii) Transgenic expression of a DHR-1 deletion mutant of MBC in *mbc*-null flies fails to rescue myoblast fusion. Abbreviations: FC, fusion competent myoblast; F, founder myoblast. **(c)** Dock7 is required for axon specification in developing hippocampal neurons. In stage 1 neurons, Dock7 is freely distributed at the cell membrane. During budding of multiple dendrites at stage 2 of neuron development, Dock7 accumulates in an asymmetric manner at the base of the dendrite that will become the axon. Interestingly, depletion of Dock7 by siRNA blocks axon specification whereas overexpression of Dock7 promotes the specification of multiple axons. At stage 3, Dock7 is found located along the axon shaft and

also at the base of the growth cone. Blocking of the formation of $\text{PtdIns}(3,4,5)P_3$ by pharmacological PI 3-kinase inhibitors prevents the Dock7-mediated axon specification.