CZH proteins: a new family of Rho-GEFs

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Summary

The Rho family of small GTPases are important regulators of multiple cellular activities and, most notably, reorganization of the actin cytoskeleton. Dbl-homology (DH)-domain-containing proteins are the classical guanine nucleotide exchange factors (GEFs) responsible for activation of Rho GTPases. However, members of a newly discovered family can also act as Rho-GEFs. These CZH proteins include: CDM (Ced-5, Dock180 and Myoblast city) proteins, which activate Rac; and zizimin proteins, which activate Cdc42. The family contains 11 mammalian proteins and has members in many other eukaryotes. The GEF activity is carried out by a novel, DH-unrelated domain named the DOCKER, CZH2 or DHR2 domain. CZH proteins have been implicated in cell migration, phagocytosis of apoptotic cells, T-cell activation and neurite outgrowth, and probably arose relatively early in eukaryotic evolution.

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Introduction

The Rho family of small GTPases includes the products of 22 human genes, the best-known members being RhoA, Rac1 and Cdc42 (Wennerberg and Der, 2004). Rho proteins are important regulators of multiple cellular activities. These include control of cell morphology, polarity, migration, adhesion to extracellular matrix proteins or other cells, proliferation, apoptosis, tumorigenesis, phagocytosis, vesicular transport and transcription (Aznar et al., 2004; Erickson and Cerione, 2004; Etienne-Manneville and Hall, 2002). Rho proteins are key regulators of cytoskeleton reorganization, controlling actin polymerization and microtubule dynamics. For example, Rac activation induces actin polymerization and integrin focal complex assembly at the cell periphery, leading to formation of lamellipodia. Rac is preferentially activated at the leading edge of migrating cells and its activity is crucial for cell migration (Etienne-Manneville and Hall, 2002). Rac also regulates phagocytosis, probably by mediating changes in the cytoskeleton (Chimini and Chavrier, 2000).

Rho proteins are active when bound to GTP and inactive when bound to GDP. Conversion of the GDP-bound proteins to the active state is catalyzed by guanine nucleotide exchange factors (GEFs). The classical GEFs for Rho GTPases share a common motif, the Dbl-homology (DH) domain, which mediates nucleotide exchange (Cerione and Zheng, 1996). In mammals, 69 DH-domain-containing proteins have been identified, illustrating the need for selective activation of Rho proteins by different signaling pathways under diverse conditions (Rossman et al., 2005; Schmidt and Hall, 2002). Until recently, DH-domain-containing proteins were considered to be the universal Rho-GEFs in eukaryotes. We review here a non-conventional Rho-GEF protein family whose members lack the DH domain and instead possess a novel form of GEF domain.

Identification of a new family of GEFs for Rho proteins

New Rho-GEFs

GEFs are distinguished from other GTPase-interacting proteins by their preferential binding to nucleotide-free (nf) GTPases compared with the GDP- or GTP-bound forms (Cherfils and Chardin, 1999; Hart et al., 1996). Dock180 was cloned in 1996 as a binding partner for the adaptor protein Crk (Hasegawa et al., 1996). The *Caenorhabditis elegans* ortholog of Dock180, Ced-5, was identified as a protein required for cell migration and phagocytosis (Wu and Horvitz, 1998), whereas the *Drosophila* ortholog, Myoblast city, was identified as a protein essential for myoblast fusion and dorsal closure (Erickson et al., 1997). The Ced-5, Dock180 and Myoblast city (CDM) proteins lack DH domains but indirect observations have indicated that they activate Rac and associate preferentially with its nf form (Grimsley et al., 2004; Hasegawa et al., 1996; Kiyokawa et al., 1998a; Kiyokawa et al., 1998b; Namekata et al., 2004; Nishihara et al., 1999; Reddien and Horvitz, 2000). This finding has implicated them as either adaptors that recruit Dbl proteins or novel Rac GEFs. More recent work has shown that CDM proteins directly interact with Rac through a newly identified GEF domain (Brugnera et al., 2002; Cote and Vuori, 2002). Parallel studies have revealed that zizimin1, cloned in a search for Cdc42 GEFs in fibroblasts, also lacks a DH domain but activates Cdc42 and selectively binds nf Cdc42 (Meller et

al., 2002). Zizimin1 shares sequence similarity with CDM proteins in two specific regions: CZH1 and CZH2 (Fig. 1). The CZH2 domain (also called the DOCKER or DHR2 domain) is the new GEF domain.

Structure and subfamilies

Database homology searches using zizimin1 and Dock180 reveal that they are members of a novel family present in a wide variety of eukaryotes that we refer to as the CZH proteins (Fig. 1, Table 1, Table 2). CZH proteins are large polypeptides that have 1800 to >3100 residues. Most members identified have both CZH1 and CZH2 domains, the CZH1 domain always preceding the CZH2 domain (Fig. 1). The exceptions are CZH proteins in budding yeasts and the *Dictyostelium* protein DdDocC, which have just the CZH2 domain (Fig. 1). The two domains might therefore be functionally linked and, indeed, our data indicate that the zizimin1 CZH1 domain binds to the CZH2 domain and inhibits the interaction with Cdc42 (N.M. et al., unpublished). Note that the phylogenetic trees constructed by comparison of CZH1 and CZH2 sequences are similar (Fig. 2). This argues that the CZH1 and CZH2 domains have generally not moved laterally between proteins during evolution but have changed gradually in the context of full-length proteins. The Dock180 CZH1 domain was recently shown to bind phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] and mediate targeting of Dock180 to the leading edge (Cote et al., 2005). Dock180 might therefore utilize its CZH2 and CZH1 domains to mediate Rac activation downstream of phosphoinositide 3-kinase (PI3K) in directed cell migration.

On the basis of domain structure, sequence similarity and phylogenetic analysis, the CZH family of proteins can be divided into four subfamilies. Almost all CZH proteins can be broadly categorized as either zizimin-related or Dock180-related proteins, because most of their sequences outside the CZH1 and CZH2 domains have similarity to one of these two proteins (Fig. 1). The zizimin- and Dock180-related proteins can each be further divided, resulting in four subfamilies: the zizimin, zir (for 'zizimin-related'), Dock180 and Dock4 proteins (Figs 1 and 2, Table 1). Proteins within these subfamilies exhibit 50-65% identity in amino acid sequence.

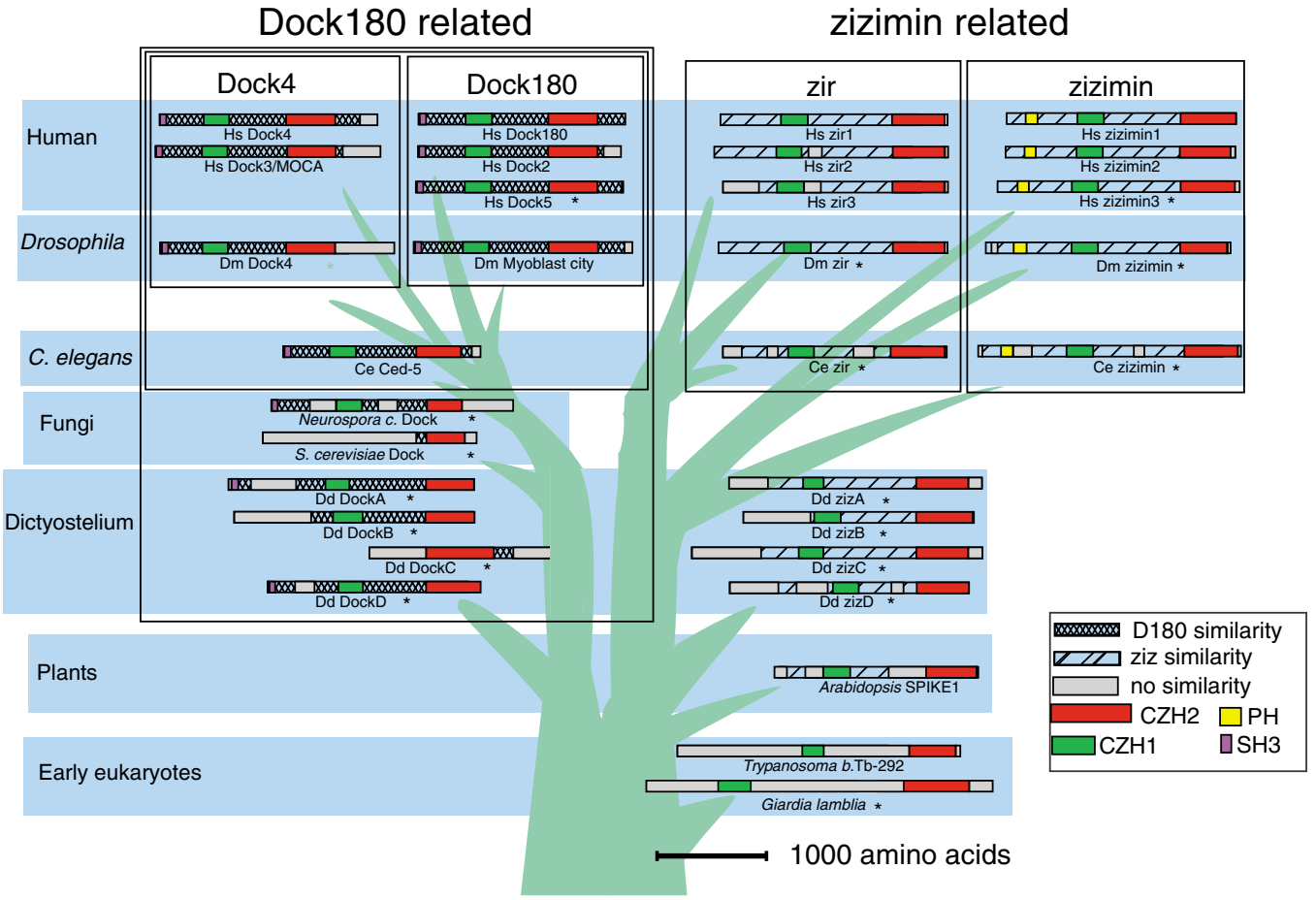

Fig. 1. Schematic structure of CZH proteins. The CZH1 (CDM-zizimin homology 1), CZH2 (CDM-zizimin homology 2), SH3 (Src homology 3) and PH (pleckstrin homology) domains are displayed. Areas outside the CZH1 or CZH2 domains with similarity to zizimin1 or Dock180 are hatched or crossed, respectively. Sequences obtained by computational translation are marked by asterisks. The tree represents the hypothetical evolution of the proteins based on sequence similarity. Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; *Neurospora c.*, *Neurospora crassa*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *Trypanosoma b.*, *Trypanosoma brucei*.

Table 1. CZH protein subfamilies and specificities

Subfamily	Human members (alternative names) [GenBank GI no.]	Comments	Substrate	*Drosophila* ortholog [GenBank GI no.]	*C. elegans* ortholog [GenBank GI no.]	Refs
Dock180	Dock180 (Dock1) [4503355]	Broadly distributed but absent from lymphocytes	Rac1*	Myoblast city [7511969]	Ced-5 [7511497] Selective interaction with Rac	Akakura et al., 2004; Brugnera et al., 2002; Cote and Vuori, 2002; Hasegawa et al., 1996; Kiyokawa et al., 1998a; Nishihara et al., 1999
	Dock2 [31377468]	Selective expression in hematopoietic cells; lacks proline-rich motifs for Crk binding	Rac1*, Rac2*			Akakura et al., 2004; Cote and Vuori, 2002; Fukui et al., 2001; Nishihara, 1999; Nishihara et al., 1999; Nishihara et al., 2002b; Sanui et al., 2003a
	Dock5 [45439362]	Dock5 has not been cloned	Unknown			
Dock4	Dock4 [29568109]	Expressed predominantly in skeletal muscle, prostate and ovary	Rac	Dm Dock4 [28381487]		Lu et al., 2005; Yajnik et al., 2003
	Dock3 (MOCA; PBP) [23297197]	Expressed predominantly in neuronal cells in the brain and spinal cord	Rac*			De Silva et al., 2003; Grimsley et al., 2004; Namekata et al., 2004
Zizimin	Zizimin1 (Dock9; KIAA1058) [22038159]	Enriched in multiple tissues of non-hematopoietic cells	Cdc42†	Dm zizimin	Ce zizimin [17506759]	Cote and Vuori, 2002; Meller et al., 2002; Nishikimi et al., 2005
	Zizimin2 (Dock11) [40068509]	Expressed predominantly in lymphocytes	Cdc42†			Nishikimi et al., 2005
	Zizimin3 (Dock10; KIAA0694) [32469767]	Expressed in some hematopoietic and non-hematopoietic tissues; not fully cloned	Unknown, low-affinity interaction with Cdc42, TCL and RhoA, but not Rac or TC10			Nishikimi et al., 2005
Zir	Zir1 (Dock6; KIAA1395) [32469768]	Zir1 has not been cloned	Unknown	Dm zir [22945582]	Ce zir [17567793]	
	Zir2 (Dock7;KIAA1771)	Zir2 has not been fully cloned	Unknown			Cote and Vuori, 2002
	Zir3 (Dock8; FLJ00346) [44889960]		Unknown			Ruusala and Aspenstrom, 2004

*Specific interaction with regard to Cdc42 or Rho. †Specific interaction with regard to Rac, Rho, TC10 and TCL.

Table 2. Additional CZH proteins

	Name	Species	GenBank GI no.	Length	Comments
Early eukaryotes		*Giardia lamblia*	29250065	3123	A Rac protein in *Giardia lamblia* (GI: 29249145)*
		Giardia lamblia	29250082+ 29250083	?	
	Tb-292	*Trypanosoma brucei*	1078707	2550	Transmembranal protein (Lee et al., 1994). A Rho protein (TcRho1) in *Trypanosoma cruzi*
		Leishmania major	70799546	2955	*
Plants	SPIKE1	*Arabidopsis thaliana*	18496703	1830	
		Oryza sativa (rice)	34902156	1852	*
Amoeba	Dd ZizA	*Dictyostelium discoideum*	66820478	2284	*
	Dd ZizB	*Dictyostelium discoideum*	66800771	2082	*
	Dd ZizC	*Dictyostelium discoideum*	19569943	2621	*
	Dd ZizD	*Dictyostelium discoideum*	66827367	2162	*
	Dd DocA	*Dictyostelium discoideum*	66801748	2221	*
	Dd DocB	*Dictyostelium discoideum*	60474615	2176	*
	Dd DocC	*Dictyostelium discoideum*	66801673	1728	*
	Dd DocD	*Dictyostelium discoideum*	66809471	1924	*
Fungi		*Neurospora crassa*	32418746	2182	*
		Cryptococcus neoformans	57229287	2117	*
		Aspergillus nidulans	40747406	2132	*
		Magnaporthe grisea	38105229	946	*
		Gibberella zeae	42551283	2030	*
		Ustilago maydis	46096729	2284	*
		Candida albicans	46442962	1914	*
		Candida albicans	46442963	1768	*
		Eremothecium gossypii	45190708	1902	*
	YLR422wp	*Saccharomyces cerevisiae*	6323454	1932	Similar genes in *Saccharomyces paradoxus*, *Saccharomyces bayanus* and *Saccharomyces mikatae**

*The sequence was obtained by computational translation.

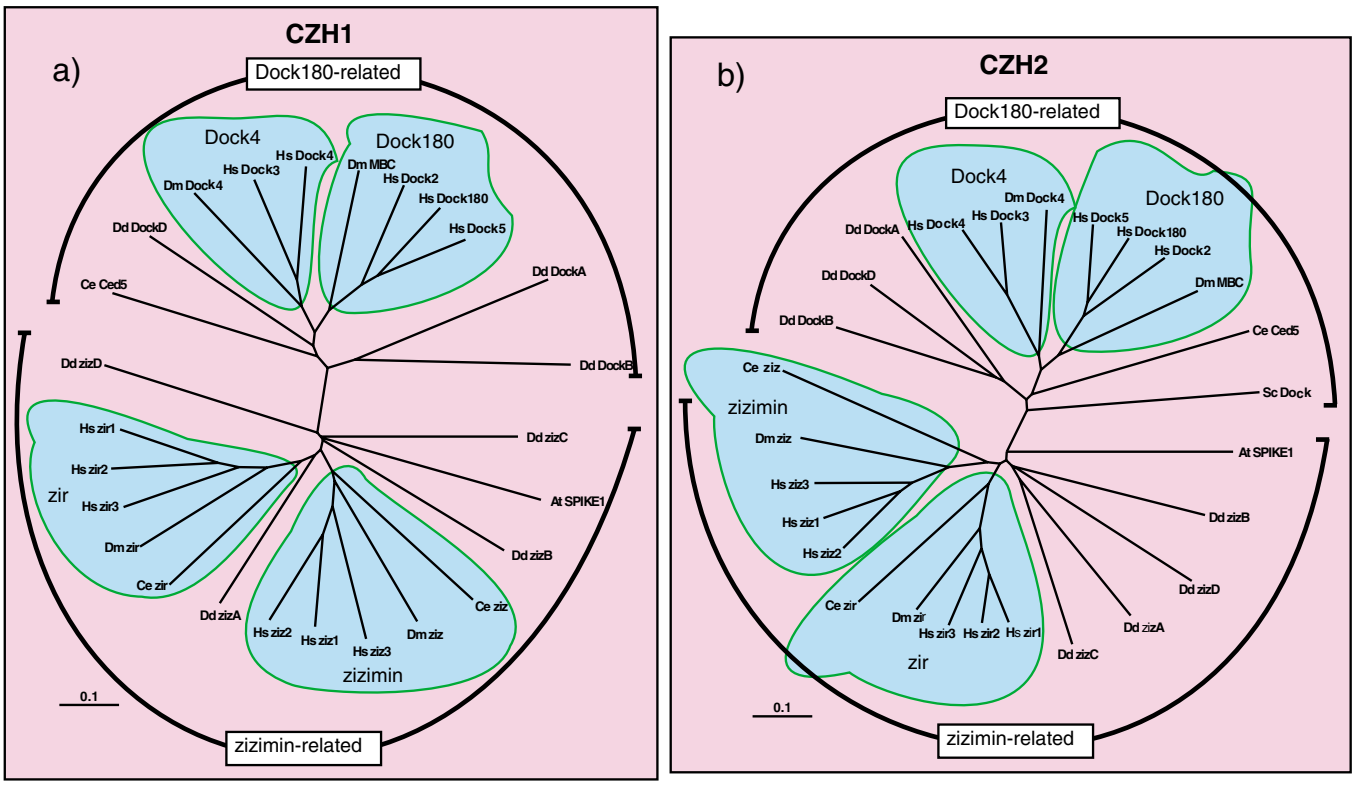

Fig. 2. Phylogenetic analysis of the CZH1 (a) and CZH2 (b) domains. Multiple alignments were built using the CLUSTALW program and the distances between all pairs of sequences in the multiple alignment were determined (Thompson et al., 1994). Phylogenetic trees were generated using the neighbor-joining method (Saitou and Nei, 1987) and trees were drawn using the TREEVIEW program (Page, 1996). Bar, 0.1 nucleotide substitutions per site. At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*.

Proteins from the zizimin subfamily have a pleckstrin-homology (PH) domain close to the N-terminus. PH domains have been implicated in targeting of proteins to membranes and in protein-protein interactions (Lemmon and Ferguson, 2000). Preliminary results indicate that the PH domain also targets zizimin 1 to membranes (N.M., M. R. Westbrook and M. A. Schwartz, unpublished). Zizimin proteins exist predominantly as dimers and the Dock180 proteins are likely to dimerize as well (Meller et al., 2004; Yin et al., 2004). Although the zir proteins lack a PH domain, they show ~30% identity to zizimin proteins along most of their sequence.

Dock180 and Dock4 proteins have an N-terminal Src homology 3 (SH3) domain and divergent C-termini containing proline-rich motifs that bind SH3 domains to mediate intra- or inter-molecular protein interactions (Mayer, 2001). The Dock180/Dock4 subfamilies are more closely related to each other compared with the zizimin/zir proteins, sharing a similar domain structure and 40% sequence identity.

The CZH2 domain: a novel GEF domain

GEF activity and selectivity for Rho protein subfamilies

Three distinct observations link the CZH2 domain to activation of Rho proteins. First, deletion or mutation of this domain abolishes GTPase activation by CZH proteins in cells (Brugnera et al., 2002; Meller et al., 2002; Namekata et al., 2004). Second, bacterially expressed CZH2 domains from zizimin1 or Dock180 proteins bind to bacterially expressed nf Rho-family GTPases, which indicates that the interaction is direct (Brugnera et al., 2002; Cote and Vuori, 2002; Meller et al., 2002). Third, CZH2 domains can catalyze nucleotide release from Rho GTPases in vitro (Cote and Vuori, 2002).

The selectivities of different CZH proteins for Rho-family GTPases, in cells and in vitro, have been tested in binding and GTPase-activation assays. The substrates for the Dock180 and Dock4 subfamilies are Rac proteins; zizimin 1 and zizimin 2 interact selectively with Cdc42; and the substrates for zizimin 3 and zir proteins remain to be identified (Table 1).

Structure of the CZH2 domain

Although the exact boundaries of the CZH2 domain remain to be determined, it is clearly very large, containing 450-550 residues (DH domains contain only ~180 residues). The sequence conservation shared by CZH2 domains is low: there is 16-17% identity in amino acid sequence between the zizimin1 and Dock180/Dock4 proteins (see supplementary material Fig. S1). This is comparable with DH domains, which share 19-29% sequence identity. Despite this, the three-dimensional structures of different DH domains are very similar (Schmidt and Hall, 2002) and this could also be the case for CZH2 domains. The *Salmonella typhimurium* SopE protein is another example of a GEF for Rho proteins that bears no DH domain (Buchwald et al., 2002; Hardt et al., 1998; Rudolph et al., 1999; Schlumberger et al., 2003). Note that there is no sequence homology between CZH2 domains and the SopE GEF domain.

Zizimin1 dimerizes through its CZH2 domain. Each dimer has two individual Cdc42-binding sites, and kinetic measurements have demonstrated increased binding affinity for Cdc42 at higher Cdc42 concentrations (Meller et al., 2004). This suggests positive cooperativity in which binding of Cdc42 to one site increases the affinity of the second site. It may represent a mechanism for regulation of GEF activity by the local GTPase concentration.

Multiple alignment of CZH2 domain sequences (see supplementary material Fig. S1) reveals stretches of similarity between the Dock180- and zizimin-related proteins along most of the domain, and the predicted secondary structures are mostly similar. Yet, there is also large variability, and the conserved areas are separated by non-conserved stretches that vary substantially in length between the two groups. Therefore, although the general functions of the CZH2 domains in the Dock180- and zizimin-related proteins are similar, some aspects of the mechanism may differ significantly.

ELMO: a Dock180 cofactor

Genetic studies in *C. elegans* have identified the CED-12/ELMO protein as an important component upstream of Rac in signaling pathways in which Rac is activated by Dock180 (Gumienny et al., 2001; Wu and Horvitz, 1998; Zhou et al., 2001). ELMO is a ~700 residues protein characterized by Armadillo repeats in its N-terminal half and PH domain, and a proline-rich motif toward the C-terminus (Debakker et al., 2004; Gumienny et al., 2001; Wu and Horvitz, 1998; Zhou et al., 2001). Although ELMO itself cannot interact with Rac, it substantially enhances Rac activation by Dock180. Indeed, ELMO and Dock180 proteins associate directly in mammals, worms and flies (Brugnera et al., 2002; Gumienny et al., 2001; Ishimaru et al., 2004; Zhou et al., 2001). The C-terminal part of ELMO is sufficient to support Rac activation by Dock180 (Grimsley et al., 2004), and engages in at least three different interactions with Dock180: (1) the ELMO proline-rich motif interacts with the Dock180 SH3 domain; (2) the ELMO PH domain interacts with the nf-Rac–Dock180–CZH2 domain complex; and (3) elements within the last 100 residues of ELMO (distinct from the proline-rich motif) interact with elements within the first 357 residues of Dock180 (distinct from the SH3 domain) (Lu et al., 2004; Lu et al., 2005; Sanui et al., 2003b). The N-terminal part of ELMO mediates its targeting to the cell membrane (Debakker et al., 2004; Grimsley et al., 2004). The ELMO-Dock180 interaction is required for Dock180-mediated Rac activation, cell migration and phagocytosis (Brugnera et al., 2002; Debakker et al., 2004; Grimsley et al., 2004; Gumienny et al., 2001; Katoh and Negishi, 2003; Lu et al., 2004; Sanui et al., 2003b; Wu et al., 2001; Zhou et al., 2001).

Three of the five mammalian Dock180-related proteins tested so far interact with ELMO. This suggests that regulation by ELMO is a general feature of the Dock180-related proteins (Grimsley et al., 2004; Janardhan et al., 2004; Sanui et al., 2003b). Zizimin-related proteins have no similarity to the N-terminal part of Dock180, which mediates ELMO binding, and therefore may not interact with ELMO.

Mammals have three ELMO proteins (ELMO1, ELMO2, ELMO3), of which two have so far been shown to interact with Dock180 proteins (Grimsley et al., 2004; Gumienny et al., 2001; Katoh and Negishi, 2003; Sanui et al., 2003b). ELMO regulates Dock180 function by several means. By binding to active RhoG, which resides at the cell membrane, ELMO can target Dock180 to the cell membrane, leading to Rac activation

(Debakker et al., 2004; Katoh and Negishi, 2003). Alternatively, the ELMO PH domain can bind to and stabilize the nf-Rac–CZH2 complex (Lu et al., 2004). Another mechanism involves the ELMO proline-rich motif. This relieves a steric inhibition within Dock180, in which the SH3 domain interacts with the CZH2 domain to block binding of Rac (Lu et al., 2005). Binding of ELMO to the SH3 domain disrupts the interaction, allowing Rac access. Experiments with Dock2 and Dock4 indicate that this mechanism might be common to the SH3-bearing Dock180-related proteins (Lu et al., 2005).

Dock180 and ELMO have been proposed to function as a bipartite GEF, and indeed the ELMO PH domain stabilizes the nf-Rac–Dock180–CZH2 complex (Brugnera et al., 2002; Lu et al., 2004). However, it is not clear whether, within the ternary complex, ELMO contacts Rac directly or acts by stabilizing a Dock180 conformation favorable for Rac binding. Dock180 mutants that do not bind ELMO possess GEF activity (Cote and Vuori, 2002; Lu et al., 2005), which suggests that ELMO in fact merely acts as a Dock180 cofactor and the mechanism for nucleotide exchange is in Dock180 per se.

Signal transduction by CZH proteins

Dock180 and its orthologs

Cell migration

Studies in mammalian cell lines suggest that Dock180 mediates activation of Rac by integrins during cell spreading and migration (Fig. 3). Binding of integrins to fibronectin (FN) leads to activation of the tyrosine kinases Src and FAK and subsequent phosphorylation of the adaptor protein $p130^{CAS}$. In turn, $p130^{CAS}$ binds to the Crk SH2 domain, which plays an important role in migration in many cellular contexts (Cary et al., 1998; Gu et al., 2001; Honda et al., 1999; Klemke et al., 1998; Li et al., 2003; Playford and Schaller, 2004; Takino et al., 2003). Adhesion to FN also induces binding of the Dock180 proline-rich motif to the Crk N-terminal SH3 domain, leading to formation of a Dock180–Crk–$p130^{CAS}$ complex, and this can recruit Dock180 to focal adhesions (Kiyokawa et al., 1998b). Accordingly, overexpression of these three proteins augments Rac activation, cell spreading and migration (Cheresh et al., 1999; Kiyokawa et al., 1998a; Kiyokawa et al., 1998b), whereas mutations in the Crk SH3 domain that abolish binding of Dock180 inhibit activation of Rac by integrins and cell migration (Gu et al., 2001; Li et al., 2003). Additionally, a dominant-negative Dock180 mutant, or knocking down Dock180 expression, inhibits Rac activation, migration and cell spreading on FN (Cote et al., 2005; Katoh and Negishi, 2003). Although early studies described CrkII as a component of this pathway, a more recent study indicates that a related protein, CrkL, might also be involved (Li et al., 2003). Note also that, in other studies, the Dock180 proline-rich motif needed for binding to the Crk SH3 domain appeared not to be required for induction of random cell migration (Grimsley et al., 2004), and interactions between Dock180 and Crk proteins that are independent of the proline-rich motif were also documented (Kiyokawa et al., 1998b; Nishihara et al., 2002a).

Laminin-10/11, vitronectin and possibly collagen also induce cell migration and Rac activation through the Dock180–Crk–$p130^{CAS}$ complex, which indicates that multiple integrins utilize this signaling pathway (Cheresh et al., 1999; Gu et al., 2001; Wu et al., 2005). Dock180 might also be linked to integrin signaling through paxillin, which binds to Dock180, probably through Crk (Valles et al., 2004). Furthermore, in response to lysophosphatidic acid (LPA), Rac can be activated by Rho through its effector mDia1. The Dock180–Crk–$p130^{CAS}$ complex is implicated in this pathway. Growth factors might therefore also utilize this signaling complex (Tsuji et al., 2002) (Fig. 3).

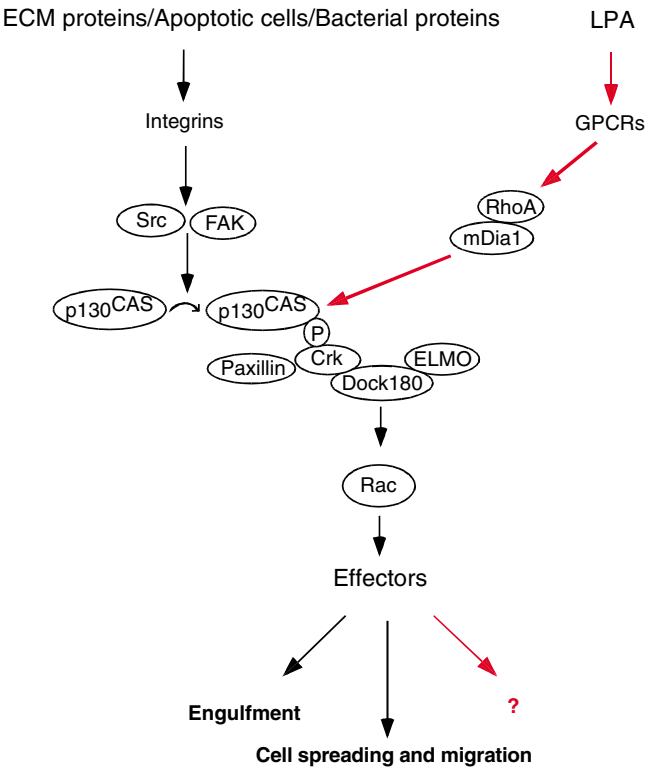

Fig. 3. Signal transduction by Dock180. Signalling pathways where Dock180 mediates Rac activation are displayed. GPCRs, G-protein-coupled receptors; ECM, extracellular matrix.

Dock180 proteins in *Drosophila* also regulate migration processes. The *myoblast city* (Mbc) mutants display defects in border cell migration during oogenesis and migration of epidermal cells that result in incomplete dorsal closure during embryogenesis and incomplete thorax closure during metamorphosis (Duchek et al., 2001; Erickson et al., 1997; Ishimaru et al., 2004; Nolan et al., 1998). The migration of border cells is guided by the platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) receptor ligand PVF in an Mbc-dependent but PI3K-independent manner (Duchek et al., 2001). The PDGF/VEGF receptor also regulates thorax closure, and Crk, ELMO, Mbc, Rac and JNK are all implicated in this pathway (Ishimaru et al., 2004).

Engulfment

Integrins also mediate engulfment of apoptotic cells or bacteria, and Dock180 is likely to play a role in this process (Fig. 3). Engulfment of apoptotic cells driven by αvβ5 integrins depends on FAK, which associates with the

cytoplasmic tail of β5 and induces Rac activation through Dock180–Crk–p130^{CAS} (Akakura et al., 2004; Albert et al., 2000; Wu et al., 2005). An additional receptor for apoptotic cells, the tyrosine kinase Mer, is involved in the process probably through activation of a Src-family kinase, which activates FAK (Wu et al., 2005). Engulfment of bacteria by mammalian cells expressing β1 integrins, which bind to the bacterial protein invasin, is likely to involve Dock180 as well (Gustavsson et al., 2004; Wong and Isberg, 2005).

Genetic studies have demonstrated that regulation of cell engulfment (and migration) processes by Dock180, Crk and ELMO proteins is conserved in *C. elegans* (reviewed by Blelloch et al., 1999; Grimsley and Ravichandran, 2003; Lehmann, 2001; Reddien and Horvitz, 2004).

The RhoG connection

Dock180 can also mediate Rac activation downstream of the small GTPase RhoG (see above). This pathway may mediate integrin-induced Rac activation, cell migration, phagocytosis and nerve growth factor (NGF)-induced neurite outgrowth (Debakker et al., 2004; Grimsley et al., 2004; Katoh and Negishi, 2003). The RhoG and Crk pathways might be connected, since Crk, Dock180 and ELMO can form a ternary complex (Gumienny et al., 2001; Wu et al., 2001), and CRK might regulate interactions between Dock180, ELMO, Rac and RhoG (Akakura et al., 2005).

Dock2

Dock2 is expressed selectively in hematopoietic cells including lymphocytes, dendritic cells and possibly others. By contrast, Dock180 is absent from lymphocytes (Fukui et al., 2001; Hasegawa et al., 1996, Akakura, 2004; Nishihara et al., 1999). Studies in knockout mice have demonstrated important roles for Dock2 in lymphocyte development, homing, activation, adhesion, polarization and migration processes (Fukui et al., 2001; Nombela-Arrieta et al., 2004; Sanui et al., 2003a) (reviewed by Reif and Cyster, 2002). Translocation of the T-cell receptor (TCR) and lipid rafts to the immunological synapse is impaired in Dock2-deficient cells (Nishihara et al., 2002b; Sanui et al., 2003a). Dock2 associates with the TCR and is required for Rac activation by the receptor. Dock2 also associates with Vav, a DH-type GEF for Rac that plays a critical role in formation of the immunological synapse and Rac activation (Nishihara et al., 2002a). Interestingly, the HIV-1 protein Nef associates with the Dock2-ELMO1 complex. This leads to Rac activation and inhibition of T-cell chemotaxis (Janardhan et al., 2004). Migration requires localized Rac activation and cell polarization; Nef could therefore potentially disturb chemotaxis by inducing global Rac activation.

Dock3 and Dock4

Dock3 (also known as MOCA) is expressed predominantly in neurons and resides in growth cones and membrane ruffles (Chen et al., 2005; De Silva et al., 2003; Namekata et al., 2004). Dock3 overexpression increases morphological complexity and the number of neurites in differentiating PC12 cells, whereas downregulation of the protein has an inhibitory effect (Chen et al., 2005). This suggests that Dock3 promotes neurite outgrowth. Dock3 expression also increases the level of N-cadherin, leading to cell-cell adhesion (Chen et al., 2005). Interestingly, mutations in Dock3 may be associated with attention-deficit hyperactivity disorder (ADHD) in humans (De Silva et al., 2003).

The gene encoding Dock4 was cloned in a screen for genes deleted during tumor progression, and in vitro and in vivo experiments have demonstrated that Dock4 possesses tumor suppressor properties (Yajnik et al., 2003). Expression of Dock4 also leads to activation of the small GTPase Rap1 and enhanced formation of adherens junctions (Yajnik et al., 2003). Rap1 activation is required for Dock4-mediated adherens junction formation, which indicates that Rap1 may mediate Dock4 tumor suppressor activity. However, expression of Dock4 in cells also leads to activation of Rac (Lu et al., 2005). Whether the Dock4 interaction with Rap1 and Rac is direct has not been tested. Given that Dock4 possesses a CZH2 domain (but not a Rap-GEF domain), and given the high sequence homology to Dock3 (54% identity), which acts on Rac, Rac activation by Dock4 is likely to be direct whereas Rap1 activation is probably indirect.

CZH proteins in *Saccharomyces cerevisiae*, *Dictyostelium* and plants

The *S. cerevisiae YLR422W* gene encodes a CZH protein, and mutations in *YLR422W* may affect filamentous growth. Importantly, the CZH2 domain of YLR422wp can bind to human Rac (Brugnera et al., 2002), which suggests that the function of the domain as a GEF for Rho proteins is conserved in yeast.

Dictyostelium possesses at least eight CZH proteins. Several of these, like *Dictyostelium* Rho proteins, are noticeably divergent from one another (Rivero et al., 2001; Rivero and Somesh, 2002) (Table 2, Figs 1 and 2). The different *Dictyostelium* CZH proteins might therefore correspond to distinct subgroups of Rho proteins. Dd zizA and Dd zizB appear to be involved in *Dictyostelium* development processes but not in chemotaxis towards cAMP (S.M. and R. Firtel, unpublished).

Green plants have Rho proteins distinct from those of other kingdoms (termed ROPs or Aracs), and these constitute a separate phylogenetic group (reviewed by Valster et al., 2000; Vernoud et al., 2003; Yang, 2002). Interestingly, there are no DH proteins in *Arabidopsis*, and the question of whether ROPs utilize GEFs, and if so which, has remained open (Valster et al., 2000). *Arabidopsis* encodes a single CZH protein, SPIKE1, that was reported recently to act as ROPs-GEF (Basu et al., 2005). Interestingly, SPIKE1 was cloned in a screen for genes controlling cytoskeletal organization, and mutations in SPIKE1 impair localized lateral microtubule clustering and polarized growth (Qiu et al., 2002). Furthermore, yet another group of ROP-GEFs has been discovered (Berken et al., 2005) – it is present exclusively in plants and uses a novel GEF domain that is unrelated to DH, CZH2 or the SopE GEF domains.

Concluding remarks

Studies conducted in recent years have established CZH proteins as non-conventional Rho-GEFs. The mechanism for nucleotide exchange by CZH proteins, and the differences

between the Dock180-related and zizimin-related proteins are not known. We hope that structural studies will shed some light on this issue. Whether ELMO is required for GEF activity in all cellular situations is another open question. Although a few biological roles of some of these GEFs have been revealed, much more is missing. Four of the eleven mammalian CZH proteins are not fully cloned, and the roles of most of the cloned members are not known. Data from CZH protein knockouts and knockdown experiments will probably address this issue in the coming years. The direct and indirect upstream regulators of CZH proteins and the downstream mediators of their GTPases are also mostly elusive. The CZH proteins studied so far activate Rac or Cdc42, and regulate actin polymerization. Could they also participate in non-actin-dependent roles of these GTPases? And do any CZH proteins act on Rho itself or other Rho-family GTPases?

DH-domain-containing proteins outnumber CZH proteins by 3–6-fold (Rossman et al., 2005). It is unclear why there are two classes of GEF and why the DH protein family expanded more over evolutionary time than did the CZH protein family. The information gathered so far suggests that principles of regulation of Dbl proteins, such as autoinhibition of GEF activity, formation of complexes with signaling proteins, and translocation within cellular compartments to induce activation, also apply to CZH proteins. Detailed kinetic studies with full-length proteins might reveal significant differences in the exchange mechanisms of DH and CZH proteins. Note also that DH-domain-containing proteins are much more versatile in their domain compositions (Schmidt and Hall, 2002). This may have provided an advantage that hooked them into different signaling pathways and contributed to their expansion.

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