

Mapping the selection mechanisms by bacterial GEFs

Zhi Huang & Jijie Chai

To cite this article: Zhi Huang & Jijie Chai (2010) Mapping the selection mechanisms by bacterial GEFs , Virulence, 1:2, 93-96, DOI: [10.4161/viru.1.2.10617](https://doi.org/10.4161/viru.1.2.10617)

To link to this article: <https://doi.org/10.4161/viru.1.2.10617>



Copyright © 2010 Landes Bioscience



Published online: 01 Mar 2010.



Submit your article to this journal [↗](#)



Article views: 79



Citing articles: 1 View citing articles [↗](#)

Mapping the selection mechanisms by bacterial GEFs

Zhiwei Huang and Jijie Chai*

National Institute of Biological Sciences; Beijing, China

Mimicry of eukaryotic signaling enzymes is a common strategy used by bacterial pathogens to manipulate host cellular signaling. The *E. coli* type III effector protein Map belongs to a large family of bacterial virulence factors that activate host Rho GTPase signaling pathways through an unknown molecular mechanism. Our recent structural study, coupled with biochemical and functional assays, establishes that this family protein, including Map, IpgB1/2 and SifA/B secreted by *E. coli*, *Shigella* and *Salmonella* respectively, acts as functional mimic of mammalian guanine nucleotide exchange factors (GEFs). Furthermore, we show that Map and its family members share a conserved mechanism with human Dbl GEFs for selection of various GTPase isoforms, revealing an evolutionary dynamic state of protein mimicry.

Rho GTPases function as bi-molecular switches and activate numerous signal transduction pathways to regulate, most importantly, actin dynamics.^{1,2} The switch function of Rho proteins depends on their cycling of two distinct conformations: a GTP-bound active state and a GDP-bound inactive state. Interconversion of these two different conformations of Rho GTPases is catalyzed by nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GEFs activate Rho proteins by promoting the exchange of GDP for GTP, whereas GAPs inactivate them by accelerating the intrinsically slow Rho GTPases' activity to generate the inactive GDP-bound form. Most of the mammalian GEFs identified so far belong to Dbl homology (DH) domain-containing proteins that are specific for distinct Rho

GTPases including RhoA, Rac1 or Cdc42. Mainly through biochemical and structural studies, the catalytic and selection mechanisms of mammalian GEFs have been well documented.¹⁻⁴ GEF-induced structural remodeling around the two conserved switch regions of Rho proteins is believed to be important for the release of GDP, whereas the variable residues, forming the 'specificity patch', from $\beta 1$, $\beta 2$ and $\beta 3$ play a crucial role in defining Rho GTPases selection by their cognate GEFs.^{3,4}

In contrast with the natural GTPase regulatory systems, bacterial pathogens may hijack GTPase communication networks by delivering GEF mimics directly into host cells.⁵ For example, the 'Type III' secreted effector SopE of *Salmonella typhimurium*, is a functional mimic of Dbl-family Rho GEF,⁶ although they share no sequence and structural homology to any Dbl proteins.⁷ Compared with the mammalian Dbl GEFs, little is known about the selection mechanism of bacterial GEF mimics. The reason for this is that there are a limited number of GEFs in the SopE family for comparison. A large family of bacterial type III effectors with a motif WxxxE (x stands for any residue) was recently found to regulate actin cytoskeletal dynamics through an unknown GTPase signaling mechanism.⁸ The prototypic family member *E. coli* Map induced cell surface filopodia, an actin-based phenotype regulated by Cdc42 (ref. 9). Other family members, that include *Shigella* IpgB1/2 and *Salmonella* SifA/B, can discriminate between cellular phenotypes by selectively regulating RhoA, Rac1 or Cdc42 signaling pathways.^{8,10,11} Map and its family members were proposed to directly mimic Rho-family GTPases.⁸ However, none of this family protein was

Key words: bacterial effector protein, mimicry, GEF, Rho GTPase, Map, SifA/B, SopE/SopE2, IpgB1/2, BopE

Submitted: 11/01/09

Revised: 11/13/09

Accepted: 11/13/09

Previously published online:

www.landesbioscience.com/journals/virulence/article/10617

*Correspondence to: Jijie Chai;
Email: chaijijie@nibs.ac.cn

Addendum to: Huang Z, Sutton SE, Wallenfang AJ, Orchard RC, Wu X, Feng Y, et al. Structural insights into host GTPase isoform selection by a family of bacterial GEF mimics. *Nat Struct Mol Biol* 2009; 16:853-60; PMID: 19620963; DOI: 10.1038/nsmb.1647.

found to be able to bind either GDP or GTP. Moreover, such a mechanism is not supported by the observations that several WxxxE-containing effectors that require host Cdc42 for formation of filopodia^{9,12} or RhoA for stress fibers.^{8,13} Our recent structural studies^{14,15} coupled with biochemical and cellular assays have shed light on the biochemical function of this enigmatic family protein.

We set out to solve the crystal structure of SifA in complex with SKIP (SifA and kinesin interacting protein¹⁶). Surprisingly, the C-terminus of SifA was shown to be a close structural homolog of SopE,¹⁴ suggesting their common activity as GEFs. Guided by this piece of structural information, we used biochemical assay to demonstrate that another member of this family protein, Map, indeed possesses *in vitro* GEF activity with a high specificity for Cdc42 (ref. 15). Single mutation (Q128Y) of the conserved residue required for GEF activity abolished the ability of Map to induce Cdc42-mediated actin-filopodia. Moreover, while the wild-type Map complemented EPECΔmap strains for producing transient filopodia in HeLa cells, the Map mutant Q128Y was unable to do so. These findings demonstrate that the *in vitro* GEF activity of Map is important for the actin dynamics propagated by a natural *E. coli* infection. To study the catalysis and selection mechanisms of Map as a GEF, we went further to determine the crystal structure of Map-Cdc42 complex.¹⁵ As expected, the structure of Map has a similar fold to that of SifA. The two conserved switch regions play a dominant role in Cdc42 recognition of Map, because they not only form hydrogen bonds with the helix $\alpha 2$, but also sandwich a loop of Map (named catalytic loop) through hydrophobic contacts. Although Map and SopE exhibit completely different catalytic architecture from Dbl proteins, they all induce remarkably similar conformational changes around the two highly conserved switch regions, particularly switch 2, following binding of Cdc42. For example, in all these three GEF-bound Cdc42, Ala59^{Cdc42} from switch 2 flips over toward the GDP binding region as seen in other GEF-GTPase complex structures, thus occluding the Mg²⁺-binding site and blocking the productive Mg²⁺ binding.

These structural observations indicate that Map, and likely its family members, integrates the mechanisms utilized by SopE and the Dbl GEFs for recognition and activation of Rho-family GTPases, respectively. Structure-based sequence alignment reveals that the $\alpha 2$ and catalytic loop residues of Map contacting the two highly conserved switch regions of Cdc42 are chemically invariable (Fig. 1), suggesting that all WxxxE effectors can interact with Rho GTPases and function as GEFs.

In addition to interacting with the conserved switch regions, two α -helices ($\alpha 4'$ and $\alpha 5$, Fig. 1) of Map also make contacts exclusively with the non-conserved residues from the specificity patch of Cdc42. Notably, these non-conserved residues of Cdc42 are smaller than their equivalents of Rac1 and RhoA. Therefore interaction of Map with the specificity patch of the latter two Rho proteins is expected to be repulsive due to steric hindrance, a prediction that was confirmed by modeling study. These data suggest that Map likely targets the specificity patch for distinguishing Cdc42 from other Rho proteins. Experimental validation of this hypothesis was demonstrated by the biochemical assay showing that substitution of four non-conserved residues from this region with their smaller equivalents in Cdc42 converted Rac1 (S41A/N43T/N52T/W56F) into a substrate of Map. A similar strategy was used to engineer Cdc42 (A41S/T43N/T52N/F56W) into a GEF of IpgB1 that is specific for Rac1. Interaction of $\alpha 4'$ and $\alpha 5$ with the specificity patch suggests that they can function as a common epitope for selection of GTPase by Map family protein. Consistent with such a role, while the residues interacting with the two switch regions of Cdc42 are conserved, those from $\alpha 4'$ and $\alpha 5$ of Map are highly variable (Fig. 1C). Careful examination of the primary sequence within these two α helices indicates that Ile156 and Phe159 from $\alpha 5$ binding to the specificity patch in Map are substituted with two basic residues (Arg142 and Lys145) in *Shigella* IpgB2 and its closely related homolog EspM1. As salt binding interaction with the specificity patch is a hallmark of Dbl-family GEFs specific for RhoA,³ IpgB2 and EspM1 are predicted to

function as GEFs specific for RhoA if they employ a similar mechanism as the mammalian Dbl GEFs for selection of different Rho GTPases. Indeed, purified IpgB2 was subsequently shown to be a selective GEF for RhoA *in vitro*. Moreover, all of the WxxxE type III effectors that activate RhoA signaling pathways in cells, including EspM1 (refs. 8 and 13), have basic residues at these equivalent sites. These results are important, because they not only further strengthen the idea that the WxxxE type III effectors can function as GEFs, but also demonstrate that these bacterial effector proteins mimic the mechanism used by host Dbl GEFs for their selection of various Rho GTPase isoforms.

One surprising aspect revealed by the structure of Cdc42-Map complex is that Map and SopE induce significantly different structural alterations in Cdc42 around the specificity patch, despite their conserved structure and remarkable similarities in guanine-nucleotide exchange mechanisms. Particularly, the highly conserved Tyr40^{Cdc42} among various Rho GTPase isoforms participates in Cdc42 recognition of SopE by contacting Ile177^{SopE} and hydrogen bonding the carbonyl oxygen of Gln194^{SopE} (ref. 7). This is unique to SopE-Cdc42 interaction, because the highly conserved Tyr40^{Cdc42} is not involved in interacting with GEFs in any known GEF-Rho GTPase complex structure. By contrast, this residue in Cdc42-Map complex rotates around its C α atom by $\sim 90^\circ$ away from the Map-Cdc42 interface. The distinct interactions of Map and SopE with Cdc42 trigger strikingly different conformational changes at the C-terminal portion of switch 1 and the specificity patch. This may suggest that SopE family protein has a different mechanism from Map family protein for selection of Rho GTPases.

So far, structures of five bacterial GEFs (Map, SifA, SopE, SopE2 (ref. 17) and BopE¹⁸) have been available. All these structures have a similar fold, featuring six α -helices that form two three-helix bundles with a “V” shape (Fig. 1A). Due to lack of statistically significant sequence homology, primary sequence alignment prior to our structural studies failed to identify the similarity between WXXXE and SopE-like proteins. However, structure-based

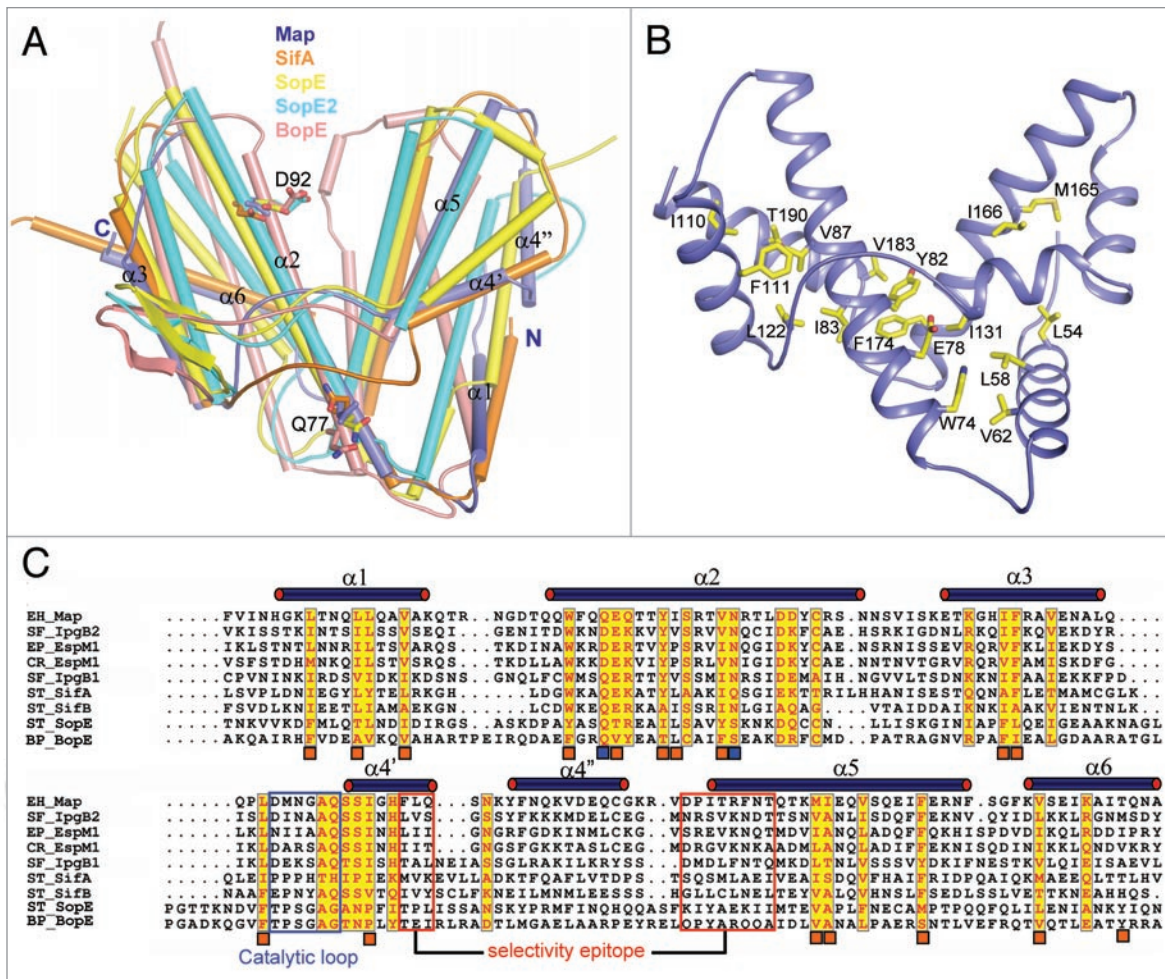


Figure 1. Structure-based sequence alignment of Map- and SopE-like proteins. (A) Structure alignment of Map (slate), SifA (orange), SopE (yellow), SopE2 (cyan) and BopE (pink). The secondary structural elements in Map are labeled. The two residues (Gln77 and D92) from $\alpha 2$ of Map as well as their equivalents in SifA, SopE, SopE2 and BopE are shown in stick. These two residues from Map and SopE form hydrogen bonds with the two switch regions of Cdc42, which are conserved in human Dbl GEFs. (B) Cartoon representation of Map structure. The side chains of those residues shown in stick and yellow are solvent-inaccessible and involved in formation of the two helix bundles. (C) Primary sequence alignment of the representing members of Map family proteins and SopE-like proteins. Similar residues are highlighted in yellow. Residues with orange squares at the bottom are those in (B) shown in stick, whereas the two residues with blue squares at the bottom are the ones forming conserved hydrogen bonds with the two switch regions of Cdc42. Residues highlighted within red frame are from the 'selectivity epitope' and blue frame from the catalytic loop. EH: *Enterohaemorrhagic Escherichia coli*; EP, *Enteropathogenic Escherichia coli*; SF, *Shigella flexneri*; ST, *Salmonella typhimurium*; CR, *Citrobacter rodentium*; BopE, *Burkholderia pseudomallei*.

alignment indicates that those residues involved in formation of the two helix bundles are primarily hydrophobic and comparatively conserved among these two family proteins (Fig. 1B and C). For example, Trp74^{Map} from the WXXXE motif, Tyr106^{SopE} and Phe110^{BopE} are similarly positioned at the juncture of the two helix bundles and completely buried (Fig. 1B), suggesting that residue at this position is important for maintaining the structural integrity of these two family proteins. By contrast, the solvent-exposed residues are highly variable (Fig. 1C). Two notable exceptions to this are Gln77 and Asp92

from the long helix $\alpha 2$ that were shown in Map to hydrogen bond with the conserved switch 2 and 1 of Cdc42, respectively. Intriguingly, similar interactions are also observed in SopE-Cdc42 and all the known structures of Rho GTPase-Dbl complexes, suggesting that interactions involving these two positions of Map are likely evolutionarily conserved. Given that those residues from $\alpha 2$ of Map are conserved in primary sequence, structure and in binding to the switch regions of Rho GTPases, they can be potentially used by bioinformatics to predict Map- and SopE-like GEFs in other bacterial pathogens, if they do exist.

References

- Jaffe AB, Hall A. Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol* 2005; 21:247-69.
- Burridge K, Wennerberg K. Rho and Rac take center stage. *Cell* 2004; 116:167-79.
- Snyder JT, Worthylake DK, Rossman KL, Betts L, Pruitt WM, Siderovski DP, et al. Structural basis for the selective activation of Rho GTPases by Dbl exchange factors. *Nat Struct Biol* 2002; 9:468-75.
- Karnoub AE, Symons M, Campbell SL, Der CJ. Molecular basis for Rac1 recognition by guanine nucleotide exchange factors. *Nat Struct Biol* 2001; 8:1037-41.
- Stebbins CE, Galán JE. Structural mimicry in bacterial virulence. *Nature* 2001; 412:701-5.
- Hardt WD, Chen LM, Schuebel KE, Bustelo XR, Galán JE. *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* 1998; 93:815-26.

7. Rossman KL, Worthylake DK, Snyder JT, Siderovski DP, Campbell SL, Sondek J. A crystallographic view of interactions between Dbs and Cdc42: PH domain-assisted guanine nucleotide exchange. *EMBO J* 2002; 21:1315-26.
8. Alto NM, Shao F, Lazar CS, Brost RL, Chua G, Mattoo S, et al. Identification of a bacterial type III effector family with G protein mimicry functions. *Cell* 2006; 124:133-45.
9. Kenny B, Ellis S, Leard AD, Warawa J, Mellor H, Jepson MA. Co-ordinate regulation of distinct host cell signalling pathways by multifunctional enteropathogenic *Escherichia coli* effector molecules. *Mol Microbiol* 2002; 44:1095-107.
10. Hachani A, Biskri L, Rossi G, Marty A, Ménard R, Sansonetti P, et al. IpgB1 and IpgB2, two homologous effectors secreted via the Mxi-Spa type III secretion apparatus, cooperate to mediate polarized cell invasion and inflammatory potential of *Shigella flexneri*. *Microbes Infect* 2008; 10:260-8.
11. Ohya K, Handa Y, Ogawa M, Suzuki M, Sasakawa C. IpgB1 is a novel *Shigella* effector protein involved in bacterial invasion of host cells: its activity to promote membrane ruffling via Rac1 and Cdc42 activation. *J Biol Chem* 2005; 280:24022-34.
12. Berger CN, Crepin VF, Jepson MA, Arbeloa A, Frankel G. The mechanisms used by enteropathogenic *Escherichia coli* to control filopodia dynamics. *Cell Microbiol* 2009; 11:309-22.
13. Arbeloa A, Bulgin RR, MacKenzie G, Shaw RK, Pallen MJ, Crepin VF, et al. Subversion of actin dynamics by EspM effectors of attaching and effacing bacterial pathogens. *Cell Microbiol* 2008; 10:1429-41.
14. Ohlson MB, Huang Z, Alto NM, Blanc MP, Dixon JE, Chai J, Miller SI. Structure and function of Salmonella SifA indicate that its interactions with SKIP, SseJ and RhoA family GTPases induce endosomal tubulation. *Cell Host Microbe* 2008; 4:434-46.
15. Huang Z, Sutton SE, Wallenfang AJ, Orchard RC, Wu X, Feng Y, et al. Structural insights into host GTPase isoform selection by a family of bacterial GEF mimics. *Nat Struct Mol Biol* 2009; 16:853-60.
16. Boucrot E, Henry T, Borg JP, Gorvel JP, Meresse S. The intracellular fate of Salmonella depends on the recruitment of kinesin. *Science* 2005; 308:1174-8.
17. Upadhyay A, Wu HL, Williams C, Field T, Galyov EE, van den Elsen JM, et al. The guanine-nucleotide-exchange factor BopE from *Burkholderia pseudomallei* adopts a compact version of the Salmonella SopE/SopE2 fold and undergoes a closed-to-open conformational change upon interaction with Cdc42. *Biochem J* 2008; 411:485-93.
18. Williams C, Galyov EE, Bagby S. Solution structure, backbone dynamics and interaction with Cdc42 of *Salmonella* guanine nucleotide exchange factor SopE2. *Biochemistry* 2004; 43:11998-2008.

©2010 Landes Bioscience.
Do not distribute.