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Mapping the selection mechanisms by bacterial GEFs

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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 β1, β2 and β3 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...
found to be able to bind either GDP or GTP. Moreover, such a mechanism is not supported by the observations that several WxExE-containing effectors that require host Cdc42 for formation of filopodia or RhoA for stress fibers. Our recent structural studies 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 kinetin interacting protein). Surprisingly, the C-terminus of SifA was shown to be a close structural homolog of SopE, 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. 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 α2, 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, Ala59Cα42 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 α2 and catalytic loop residues of Map contacting the two highly conserved switch regions of Cdc42 are chemically invariant (Fig. 1), suggesting that all WxExE effectors can interact with Rho GTPases and function as GEFs.

In addition to interacting with the conserved switch regions, two α-helices (α4' and α5, 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 α4' and α5 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 α4' and α5 of Map are highly variable (Fig. 1C). Careful examination of the primary sequence within these two α-helices indicates that Ile156 and Phe159 from α5 binding to the specificity patch in Map are substituted with two basic residues (Arg412 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 WxExE 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 WxExE 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 Tyr40Cα42 among various Rho GTPase isoforms participates in Cdc42 recognition of SopE by contacting Ile177Cα42 and hydrogen bonding the carbonyl oxygen of Gln194Cα42 (ref. 7). This is unique to SopE-Cdc42 interaction, because the highly conserved Tyr40Cα42 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 Cα atom by ~90° 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 BopE19) 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 WxExE and SopE-like proteins. However, structure-based
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, Trp74Map from the WXXXE motif, Tyr106SopE and Phe110BopE 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 α2 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-Db1 complexes, suggesting that interactions involving these two positions of Map are likely evolutionarily conserved. Given that those residues from α2 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.

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