The bacterial guanine nucleotide exchange factors SopE-like and WxxxE effectors

Running title: The SopE and WxxxE effector GEFs

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Abstract

Subversion of Rho family small GTPases, which control actin dynamics, is a common infection strategy used by bacterial pathogens. In particular, *Salmonella*, *Shigella*, enteropathogenic (EPEC) and enterohemorrhagic (EHEC) *Escherichia coli* translocate type III secretion system (T3SS) effector proteins to modulate the Rho GTPases RhoA, Cdc42 and Rac1 that trigger formation of stress fibers, filopodia and lamellipodia/ruffles, respectively. The *Salmonella* effector SopE is a guanine nucleotide exchange factor (GEF) that activates Rac1 and Cdc42, which induce ‘the trigger mechanism of cell entry’. Based on a conserved Trp-xxx-Glu motif the T3SS effector proteins IpgB1 and IpgB2 of *Shigella*, SifA and SifB of *Salmonella* and Map of EPEC and EHEC were grouped together into a WxxxE family; recent studies identified the T3SS EPEC and EHEC effectors EspM and EspT as new family members. Recent structural and functional studies have shown that representatives of the WxxxE effectors share with SopE a 3D fold and a GEF activity. In this minireview we summarize contemporary findings related to the SopE and WxxxE GEFs in the context of their role in subverting general host cell signaling pathways and infection.
Introduction

Colonization, multiplication and dissemination are the key steps of an infectious cycle. To persist within the hostile in vivo environments bacterial pathogens utilize sophisticated virulence strategies to subvert and hijack cellular and systemic functions. A common infection strategy used by Gram-negative pathogens involves injection of virulence factors, known as effectors, by the type III secretion system (T3SS), from the bacterial cell directly into the eukaryotic cell (reviewed in (26)). The injected effectors target different cellular compartments and subvert numerous signalling pathways for the benefit of the invaded or attached bacteria. Due to their essential role in regulating key cellular functions Rho-family small G proteins are common targets of T3SS bacterial effectors (reviewed in (23)).

To date, 22 members of the Rho GTPase family, belonging to the small GTPase protein superfamily, have been identified (54). Cdc42, Rac1 and RhoA, which trigger formation of filopodia, lamellipodia/ruffles and stress fibers, respectively, are the best characterized (29). The Rho GTPases share a conserved structure consisting of two flexible domains called Switch I and Switch II and a phosphate binding loop (P-loop) which together form a Mg$^{2+}$ and nucleotide-binding pocket (reviewed in (22)). The small GTPases are modified post-transcriptionally by the addition of a lipid moiety to the C-terminus (farnesyl, geranyl, palmitoyl or methyl), signalled by the carboxyl-terminal CAAX motif (55), which targets them to different membranous compartments.

The function of the small GTPases is strictly regulated. By binding the two switch domains and the lipid moiety, the Guanine nucleotide Dissociation Inhibitors (GDIs) prevent membrane localization and maintain the GTPases in an inactive state in the cytosol (35, 59). Small GTPases act as molecular switches cycling between GTP bound (active) and GDP bound (inactive) conformations. Switching a GTPase on is mediated by Guanine nucleotide
Exchange Factors (GEFs) that facilitate exchange of GDP for GTP. The intrinsically low GTP hydrolysis activity of Rho GTPases is greatly increased by interaction with GTPase-Activating Proteins (GAPs), leading to recycling of the G protein to its GDP-bound, inactive form. The Rho GTPases transmit signals in a GTP-dependent manner by activating and/or recruiting downstream effector proteins to their sites of action (reviewed in (38)).

Rho GTPases GEFs belong mainly to the Dbl family and contain a catalytic Dbl homology domain (DH) and a pleckstrin homology domain (PH), which mediates membrane association, modulates the activity of the DH domain and probably determines substrate specificity (39). The DH domain is involved in the exchange of the GDP to GTP by a mechanism called “push and pull”. It interacts specifically with the Rho GTPases Switch I and Switch II regions leading to a conformational change and ejection of GDP and Mg\(^{2+}\). This in turn leads to loading with GTP, which is present in the cytosol at a high concentration (72). Loading of Rho GTPase with GTP-Mg\(^{2+}\) triggers the dissociation of the GEF Rho GTPase complex and allows interaction of the latter with its effectors.

In order to subvert the Rho GTPase pathways, pathogenic bacteria inject into eukaryotic cells effector proteins that mimic GEF, GAP, or RhoGDI. For example Salmonella inject equivalent amounts of the T3SS effector proteins SopE and SptP that control the activity of Cdc42 and Rac1. Although sharing no sequence or structural similarities with eukaryotic GEFs (56), SopE triggers nucleotide exchange, which induces ‘the trigger mechanism of cell entry’ (32). SopE is then poly-ubiquitinated and targeted to the proteosome before SptP, which has a GAP activity and exhibits much slower degradation kinetics, helps the cell to recover from the SopE-induced membrane ruffling (42).

Recently, Alto et al. (1) assembled several known T3SS effectors into a single family that share the common motif Trp-xxx-Glu (WxxxE). This family originally included the
Salmonella SifA and SifB, the Shigella IpgB2 and IpgB1 and the enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) Map. Since the original classification new family members, EspM (3) and EspT (16), were discovered in EPEC, EHEC and *Citrobacter rodentium* (Fig. 1). Although initially thought to be Rho GTPase mimics (1), recent structural, biochemical and functional data show that the WxxxE effectors, like SopE, are Rho GTPases GEFs, which are the focus of this review.

**The WxxxE effectors: from Rho GTPase mimics to Rho GTPase GEFs**

*The Rho GTPase mimicking hypothesis*

In 2006 Alto *et al.* have shown that when expressed ectopically several of the WxxxE effectors subvert actin dynamics (1). IpgB2 induced formation of stress fibers, Map induced filopodia and IpgB1 induced formation of membrane ruffles, phenotypes which correspond to those induced by the activated forms of RhoA, Cdc42 and Rac1 respectively (Fig. 2). In order to determine if formation of stress fibers induced by IpgB2 was epistatic to RhoA, the activity of endogenous RhoA was inhibited either by dominant negative or by the bacterial toxins YopT and C3. In these conditions, IpgB2-induced stress fiber formation was not affected, suggesting that IpgB2 was acting independently of the small GTPase. Concordantly, IpgB2 was shown to interact with the Rho coiled coil p160 (ROCK) serine/threonine kinase and the RhoA binding motif of the formin mDia, the two canonical RhoA effectors involved in stress fiber formation (1). *In vitro* ROCK kinase assays showed that IpgB2 directly stimulated the activity of ROCK. Furthermore, inhibition of ROCK by the chemical inhibitor Y-27632 abolished IpgB2-induced stress fiber formation (1). Together these findings led Alto and colleagues to conclude that IpgB2 acts as a RhoA mimic, triggering the RhoA pathway by directly activating the RhoA effectors ROCK and mDia. This mechanism was extended to other WxxxE family members and in the absence of sequence similarities and structural data.
the results from the seminal paper of Alto et al (1) supported the hypothesis that the WxxxE effectors function as small GTPases mimics.

Before the WxxxE effectors were grouped together, Ohya et al. showed that IpgB1 induces formation of membrane ruffles which was dependent on Rac1 and to a lesser extent on Cdc42 (52). A follow-up study by Handa et al. has shown that IpgB1 binds the N-terminal region of the engulfment and motility protein (ELMO) in a similar manner to RhoG (30). Formation of membrane ruffles by IpgB1 was dependent on the ability of ELMO to bind the atypical Rac1 GEF Dock180. Cells transfected with truncated ELMO, which cannot bind Dock180, or Dock180, which cannot bind Rac1, failed to induce formation of IpgB1-dependent membrane ruffles upon Shigella infection (30). Translocation of ELMO-Dock180 complex to the membrane is mediated by activated RhoG. Likewise, expression of IpgB1 induces translocation of the ELMO-Dock180 complex to the membrane where it induces membrane ruffles (30). Together these results led the authors to conclude that IpgB1 activates Rac1 by binding the ELMO-Dock180 bipartite GEF (30). These results extended the mimicking paradigm as Handa et al. have shown that IpgB1 was mimicking RhoG (30).

The ability of Map to induce formation of filopodia at the EPEC attachment sites during the early stages of infection was originally described by Kenny and co-workers in 2002 (41). Using dominant negative constructs Kenny et al. showed that Map induces filopodia in a Cdc42-dependent but Rac1-independent manner. These results were confirmed by Berger et al. that furthermore demonstrated that Map induces activation of Cdc42 (7). Similarly to IpgB2, transfection or translocation of the EspM effectors resulted in formation of stress fibers in a ROCK-dependent manner (3) (Fig. 2). Production of stress fibers by EspM required active RhoA, since inhibition of this GTPase by dominant negative constructs abolished stress fiber formation (3). Moreover, pull-down experiments have shown that expression of EspM induced activation of RhoA (3). EspT induces formation of membrane
ruffles, similar in morphology to those induced by IpgB1, and lamellipodia (Fig. 2) (16), that facilitate EPEC invasion (15). The function of EspT was dependent on cellular GTPases as EspT enriches the GTP bound cellular fractions of Rac1 and Cdc42 in in vitro pull-down assays, while inhibition of Rac1, and to a lesser extent Cdc42, blocked EspT-induced formation of membrane ruffle and lamellipodia (16) and invasion (15). Despite the similarity between the phenotypes induced by EspT and IpgB1 the mechanism by which membrane ruffles and lamellipodia are formed by these two WxxxE effectors is distinct as EspT induces cytoskeletal re-arrangements independently of ELMO or Dock180 (16, 30); these results are consistent with the low sequence similarity between IpgB1 and EspT (Fig. 1). Moreover, siRNA and dominant negatives showed that formation of membrane ruffles by EspT is dependent on the Rac1 effector Wave2 (15).

WxxxE structure and function

The publication of the crystal structure of SifA in complex with the PH domain of its effector SKIP (SifA and kinesin–interacting protein) (51), represents a turning point in our understanding of the WxxxE effectors. SifA and SifB, which contrary to the other WxxxE effectors do not appear to affect actin dynamics, are particular in that they consist of two domains (51). Ohlson et al. have shown that while the N-terminal domain of SifA binds SKIP, the C-terminal domain, harbouring the WxxxE motif, contains two three helix bundles that form a V shape structure (51). This structure is surprisingly highly similar to that of SopE (14). The crystal structure of Cdc42 in complex with the biologically active catalytic fragment of SopE (residues 78-240) highlighted the mechanism by which SopE mediates guanine nucleotide exchange (32, 14) and as a result formation of membrane ruffles (Fig. 2). Although SopE does not share sequence or structural similarity with known eukaryotic GEFs, it locks the Switch I and II regions of Cdc42 in the same conformation as that observed in the equivalent regions of Rac1 or Cdc42 bound to the eukaryotic GEFs Tiam or Dbs, respectively.
SopE is composed of six α-helices forming two three-helix bundles arranged in a V-shaped fashion. Connecting the two arms of the V is a small two-stranded β-sheet followed by a peptide segment harbouring a GAGA motif. Binding of SopE induces conformational changes in the Switch regions of Cdc42, which are mostly due to insertion of the catalytic GAGA motif between Switch I and Switch II. This leads to exertion of a push and pull type movement, which induces the release of the guanine nucleotide. Consistently, replacement of the first glycine of the GAGA motif by alanine abolishes SopE activity in vitro (14). The two GEF homologues of SopE, SopE2 (Salmonella) and BopE2 (Burkholderia pseudomallei) share with SopE a similar catalytic loop sequence and structure, suggesting a common nucleotide exchange mechanism (5, 65, 66, 70). Interestingly, several of the Cdc42-interacting residues of SopE are conserved in SifA. Moreover, although not similar in sequence, the chemical properties of the residues within the putative catalytic loop of SifA are comparable to those found in SopE (51). Nonetheless, despite the similarities with SopE and the ability to bind RhoA no GEF activity has yet demonstrated for SifA (4, 51).

Two recent independent reports have shown that several WxxxxE effectors are RhoGTPase GEFs (4, 36). First, Huang et al. demonstrated that Map specifically binds nucleotide free Cdc42 and is able to induce release of GDP and incorporation of GTP (36). Moreover, the crystal structure of Map in complex with Cdc42 showed a similar structure to that of SopE and SifA, which is composed of seven α-helices arranged into one three-helix bundle and one four-helix bundle forming a V-shaped structure; a putative catalytic loop connects the two V-shaped arms. Furthermore, comparing the complexes of Cdc42 with Map, SopE, and the human GEF ITSN1 revealed that despite lacking sequence homology these three GEFs induce similar conformational changes in Cdc42, suggesting a common mechanism of nucleotide exchange. Importantly, although the sequences of the catalytic loops are somewhat different between SopE and Map, it was shown that an alanine and a glutamine residues
cement the interaction of Map with the Switch I region of Cdc42 by making a hydrophobic contact and a hydrogen bond, respectively (36). Concordantly, deletion of the catalytic loop or substitution of either of these two residues inhibited the GEF activity of Map in vitro and compromised host signalling functions of Map during infection (36). Furthermore, GEF activity assays using recombinant IpgB1 and IpgB2 have shown that the former triggers nucleotide exchange in Rac1 and to a lower extent in Cdc42 (but not in RhoA) while the latter induces strong activation of RhoA and weak activation of Cdc42 and Rac1 (36). This Rho GTPase specificity of the WxxxE effectors appears to be governed by a complementary paring between residues at the α4-α6 “selective epitope” of the effectors and the β2-3 interswitch strand of the GTPases.

In a parallel study, EspM2 has been shown to bind tightly and irreversibly to RhoA in vitro and to specifically facilitate loading of GTP into purified RhoA (4). Moreover, modelling the structure of EspM2 aided by NMR, showed that it also adopts a SopE-like conformation (4). As it was shown for Map, mutagenesis of the equivalent glutamine within the catalytic loop impaired the ability of EspM2 to induce loading of GTP into RhoA and formation of stress fibers during ectopic expression (4).

Alto et al. have shown that substituting the tryptophan or glutamic acid residues within the WxxxE motif with alanine was detrimental for function (1). As conservative substitutions (replacing the tryptophan and glutamic acid residues with tyrosine and aspartic acid respectively) in EspM2 did not result in any significant loss of function (3), the conserved W and E residues were hypothesized to have mainly a structural role. Indeed, the 3D structures of SifA and Map and the EspM2 model have shown that the W and E residues are positioned around the junction of the two three-helix bundles, maintaining the conformation of the putative catalytic loop through hydrophobic contacts with surrounding residues (36, 51). Consistently, an EspM2 W70A was highly unstable (4). Interestingly, although sharing
similar 3D structures with the WxxxE effectors, SopE has neither a W nor an E equivalent (Fig. 3). Nonetheless, taken together these results show that the WxxxE effectors belong to a large family of bacterial Rho GTPase GEFs, which also includes SopE, SopE2 and BopE2.

Regulation of the WxxxE effector activities

As it is now apparent that the WxxxE effectors, as is SopE, are Rho GTPase GEFs, there is a need to discuss this activity in the context of previously published data. For example Alto et al. (1) have shown that IpgB2 interacts with the RhoA effectors ROCK and mDia. It is possible that by binding simultaneously the Rho GTPase and its effectors IpgB2 amplify the transmitted signal, as was shown for eukaryotic GEFs (37). This might suggest a mechanism of nucleotide exchange by the WxxxE effectors involving formation of a tripartite complex consisting of GEF-Rho GTPase-effector as was suggested for EspM2 (4). Handa et al. have shown that IpgB1 forms a complex with ELMO-Dock180, which is essential for activation of Rac1 in vivo (30). Furthermore, Map contains a carboxy terminal PSD-95/Disk-large/ZO-1 (PDZ)-binding motif (DTRL), which mediates interaction between Map and PDZ1 of the scaffold protein sodium/hydrogen exchanger regulatory factor-1 (NHERF1) (1, 63). Alto et al. have shown that depleting cells of NHERF1 abolished Map-induced filopodia formation (1) while Simpson et al. (63) have shown that cells infected with EPEC expressing Map_{sTRL} exhibited no filopodia at 30 min post infection. Recently, Berger et al. have shown that maintenance of filopodia on the cell surface is dependent on the Map PDZ-binding motif, ezrin and the RhoA/ROCK pathway (7). These results suggest that the intrinsic GEF activity of these effectors is regulated by formation of large signalling complexes, as was also described for few eukaryotic GEFs, which were linked to activation of Rho GTPases in distinct cellular localizations and pathways (6, 60, 71).
Role of SopE and the WxxxE effector GEFs in pathogenesis

SopE and the WxxxE effectors are central to the EPEC, EHEC, *Shigella* and *Salmonella* infection as they control cell invasion, intracellular survival and modulation of the host immune responses (Summarized in Fig. 4). Most of these activities are dependent on the GEF activity of these effectors.

*SopE and the WxxxE effectors - invasion and intracellular survival*

The virulence strategy used by *Shigella* and *Salmonella* involves invasion of enterocytes and macrophages. To invade non-phagocytic cells *Shigella* and *Salmonella* use their T3SS to deliver effectors, which subvert actin dynamics leading to formation of membrane ruffles and macropinocytic pockets. *Shigella* invasion is dependent on T3SS effectors IpgB1, IpaC, IpaA, VirA and IpgD (49, 68, 69, 73), while *Salmonella* invasion requires several SPI-1 effectors including SopE, SopE2 and SopB (5, 32, 50). Cell invasion by *B. pseudomallei* is mediated by BopE (66).

In addition to playing a role in invasion, SopE has been suggested to affect intracellular bacterial survival by promoting fusion between the *Salmonella* containing vacuole (SCV) and early endosomes via recruitment and activation of the GTPase Rab5 (45). The SPI-2 effector SifA plays an important role in intracellular survival and virulence (8); while little is currently know of the function of SifB (9, 24). SifA contains a carboxyl-terminal CAAX motif, commonly found in the small GTPases, which when prenylated, facilitate membrane localization and activity (9, 53). SifA is involved in formation of the *Salmonella*-induced filaments (Sifs), which play a role in maintaining the integrity of the SCV (8, 58, 64). To achieve this SifA controls membrane dynamics and finely coordinates the equilibrium between the molecular motors kinesin and dynein. Indeed, by binding the host protein SKIP SifA inhibits recruitment of kinesin to the SCV (10), while by interacting with activated Rab7
it inhibits recruitment of dynein (33). The role played by the WxxxE domain of SifA in these activities is not currently known.

In contrast with the other bacteria encoding SopE or the WxxxE effectors, the attaching and effacing pathogens (A/E) EPEC, EHEC and C. rodentium were considered to be exclusively extracellular, although sporadic reports have shown that atypical EPEC strains can invade non-phagocytic cells (21, 34). This invasive ability has been linked to the adherence pattern and tight association of EPEC with the host cell membrane, which was hypothesized to produce a passive push effect leading to internalization. Recently EspT has been shown to play an analogous role during EPEC and C. rodentium infections to that of IpgB1, SopE, SopE2 and BopE by promoting invasion into non-phagocytic cells via the triggered mechanism. EPEC and C. rodentium invasion requires Rac1 and Wave2 and to a lesser extent Cdc42 (15). Invaded EPEC resides in an E. coli containing vacuole (ECV) and induces formation of intracellular actin comets which appear to promote intracellular replication/survival (15). Screening for the presence of espT among clinical EPEC and EHEC strains has shown that it is a rare effector present only in 1.8 % of the EPEC strains and missing from all the O157 and non-O157 EHEC isolates (2). EspT defines a new rare category of invasive EPEC (15).

**SopE and the WxxxE effectors - modulation of host responses**

*S. Typhimurium* is able to modulate innate immune responses in cultured epithelial cell models independently of the classical TLRs (Toll-like) or NLRs (NOD-like) receptors but via mechanisms involving SopE, SopE2, and SopB (13). By stimulating the Rho GTPases these effectors activate the MAP kinase and NF-kB signalling pathways (13). Moreover, in a Cdc42 and Rac1 dependent manner, SopE stimulates Caspase-1 activation *in vitro* which is needed for induction of mucosal inflammation *in vivo* (46). As similarly to SopE the WxxxE effectors are Rho GTPase GEFs they might also modulate innate immune response. Indeed,
activation of Rac1 by IpgB1-ELMO-Dock180 complex increases the cellular level of phosphorylated JNK, a MAP kinase involved in host responses (30). Moreover, a recent study by Fukazawa et al. (25) suggested that IpgB2, and to a minor extent IpgB1, activate NF-kB. IpgB2 was shown to activate NF-kB via a unique pathway involving GEF-H1, RhoA and NOD1.

SopE and the WxxxE effectors – subversion of intestinal barrier functions and diarrhea

Shigella, Salmonella, EPEC and EHEC diarrheal pathogens. Although other T3SS effectors are involved in disruption of the intestinal barrier functions and alteration of tight junctions (TJ), SopE and the WxxxE family members play an important role in this process.

SopE and SopE2 disrupt TJ structure and function (11). In contrast to wild type S. Typhimurium, the double sopE/sopE2 mutant did not increase the permeability of polarized epithelial cell monolayers and was unable to cause redistribution of ZO-1 and occludin, or to alter cell polarity. As these phenotypes are dependent on host protein prenylation (geranylgeranylation) it was suggested that SopE and SopE2 utilize their ability to stimulate Rho family GTPases to disrupt TJ (11).

Recently, EspM2 has been shown to play a role in the disruption of the architecture of the polarized epithelial monolayer (62). Translocation or ectopic expression of EspM2 affected localization of ZO-1 that migrated towards the basal side of the polarized epithelial monolayer. This in turn led to cell rounding and extrusion form the monolayer. However the TJ alterations induced by EspM2 did not interfere with their functionality, in fact increased TER values were observed upon EspM2 expression suggesting that this effector might play a role in maintaining TJ during infection (62).

Map has been shown to play a role in reducing transepithelial electrical resistance (TER) in polarized Caco-2 cells (17, 43), although this was not reproduced in a subsequent study (63).
Furthermore, in 2006 Dean et al. reported a role for Map, together with the T3SS effectors EspF and Tir and intimin in the effacement of absorptive microvilli and inactivation of the sodium-D-glucose cotransporter (SGLT-1), EPEC phenotypes which are directly correlated with watery diarrhea (18).

**In vivo functions of SopE and the WxxxE effectors**

Despite major advances in our understanding of SopE and the WxxxE effectors *in vitro*, a complete picture of the function of these effectors in pathogenesis is yet to be obtained. However, several *in vivo* studies provided some information about the role played by these effectors during infection.

The most dramatic *in vivo* phenotype associated with a WxxxE family member was observed with a *sifA* mutant. Consistent with the fact that SifA is required for *Salmonella* replication in macrophages *in vitro*, a *Salmonella sifA* mutant was severely attenuated during single or mixed mouse infections (8, 12, 24, 57). In contrast, a *sifB* mutant had little effect on virulence (24, 57). Using murine models of intestinal inflammation it was shown that SopE, and to a lesser extent SopE2, contribute to colitis (31) and virulence (40); SopE2 also induces diarrhoea in calves (74). It is worth noting that while SopE is absent from most of the *S. Typhimurium* isolates, SopE2 is conserved in all pathogenic strains (5, 65). Similarly to SopE, BopE has also been shown to play a role in *B. pseudomallei* virulence as *bopE* mutants were attenuated when tested in a mouse model (67).

The role of *IpgB2* and *IpgB1* in *Shigella* virulence was investigated using both the Sereny test (61) and the murine pulmonary model (44). An *ipgB1/ipgB2* double mutant was highly attenuated as it did not induce any keratoconjunctivitis and was associated with reduced mortality (28). However, no particular phenotype was associated with a single *ipgB2* mutant (28), and the ability of *Shigella* to induce keratoconjunctivitis using the Sereny test was
strongly enhanced in a \textit{ipgB1} mutant (28). The precise role of \textit{IpgB1} and \textit{IpgB2} in pathogenesis \textit{in vivo} needs to be clarified.

The role played in pathogenesis by Map, and more recently by EspM2 and EspT, has been investigated using the mouse-specific pathogen \textit{C. rodentium}, the etiological agent of transmissible colonic hyperplasia, commonly used as a model for human EPEC and EHEC infections (reviewed in (47)). Single infection studies reported a subtle role for Map in colonization of the mouse colon (19, 48), while a mixed infection of wild type and map mutant revealed that the latter is extensively out competed (48). In addition, intestinal mitochondrial structure disruption and impaired mitochondrial cellular metabolism observed following \textit{C. rodentium} infection were shown to be Map-dependent (43). Map was reported to have a role in TER disruption \textit{in vivo} (43), although it had no role in maintaining colon water content at day 7 post infection (27). However, an independent study which compared stools water content in mice colonized at similar levels by wild type \textit{C. rodentium} and a map mutant showed higher water content in the former group over a 12 days infection course, suggesting a possible role of Map in diarrhoea (63). This phenotype is consistent with binding of Map to NHERFs (63), which regulate ion channels activities. Recently, mouse infections have shown that while a \textit{C. rodentium} \textit{espT} mutant did not exhibit a significant difference in terms of bacterial shedding compared to wild type \textit{C. rodentium}, an \textit{espM2} mutant was shed at significantly lower levels, and caused less mortality in the highly susceptible CH3/HeJ mouse strain (20).

\textbf{Conclusions and perspectives}

SopE and the WxxxE effectors constitute an example of convergent evolution in bacterial virulence. Although exhibiting little sequence homology the WxxxE effectors and SopE share similar structure and function. Their mechanism of nucleotide exchanges appear to be similar to that employed by eukaryotic GEFS. While WxxxE effectors can trigger nucleotide
exchange *in vitro*, once translocated it appears that they function in the context of large signalling complexes, which are likely to regulate their activity. Importantly, while sharing 3D structure with the other family members and capable of binding RhoA (4, 51), no GEF activity was thus far associated with SifA. Extensive research over many years has shown that SopE and the WxxxE effectors are involved in diverse aspects of infection, including cell invasion, intracellular survival and replication, modulation of host responses and subversion of the intestinal barrier function (Fig. 4). Contrasting the rapid characterization of the *in vitro* phenotypes of SopE and the WxxxE effectors, our understanding of their role in virulence is lagging. Translating the fascinating cell biology surrounding these effectors to pathogenesis is the challenge of future studies.
Acknowledgements

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References


of the type III secretome of *Citrobacter rodentium*. J. Biol. Chem. Published on December 24, 2009 as Manuscript M109.086603


to mediate polarized cell invasion and inflammatory potential of *Shigella flexenri*. Microbes Infect. **10:**260-268.


68. **Tran Van Nhieu, G., A. Ben-Ze'ev, and P. J. Sansonetti.** 1997. Modulation of bacterial entry into epithelial cells by association between vinculin and the *Shigella* IpaA invasin. ENBO J. **16:**2717-2729.


fold and undergoes a closed-to-open conformational change upon interaction with Cdc42. Biochem. J. 411:485-493.


**Figures legends**

**Figure 1. SopE and the WxxxE effectors.** Multiple sequence alignment with hierarchical clustering of IpgB1 and IpgB2 from *S. flexneri* 2a, EspM2 from EHEC O157:H7, EspT from *C. rodentium*, Map from EPEC E2348/69, the C-terminal, WxxxE-containing motif of SifA and SifB, and SopE and SopE2 from *S. Typhimurium* and BopE from *B. pseudomallei*. Similar and identical residues are highlighted in grey. The WxxxE motif and the catalytic loops are boxed.

**Figure 2. SopE and WxxxE effectors subvert actin dynamics.** Serum-starved Swiss 3T3 cells were mock transfected or transfected with the mammalian expression vector pRK5 encoding myc-tagged IpgB2, EspM2 (not shown), Map, IpgB1, EspT or the catalytic domain of SopE (residues 78-240) for 16 h. Ectopic expression of IpgB2 and EspM2 induced formation of stress fibers, Map induced filopodia formation, IpgB1 induced formation of membrane ruffle while EspT and SopE induce membrane ruffles and lamelipodia. Actin was stained with Oregon Green phalloidin, the myc tag was detected with monoclonal antibody.

**Figure 3: Structure of SopE and the WxxxE effectors.** A. Overlay of the crystal structure of Map (yellow; pdb:3gcg; (36)), the crystal structure of the WxxxE-containing domain of SifA (green; pdb:3cxb; (51)), the model of EspM2 (purple; (4)) and the crystal structure of the GEF domain of SopE (cyan; pdb:1gzs; (14)). Although these structures generally superimpose well, there are differences between the helical arrangements within the corresponding WxxxE region of SopE (red box). Moreover differences in the orientation of the catalytic loop between SopE and Map and SifA and EspM2 could be accounted to the fact that crystal structures of SopE and Map are solved in complex with Cdc42, while the SifA
structure is of Rho GTPase free effector. EspM2 was modelled on the SifA structure. B. Within the WxxxE motif of Map E78 makes 2 hydrogen bonds via the carboxyl oxygen to the backbone nitrogen of S130 (in helix 4), whilst the ring nitrogen of W74 makes a single hydrogen bond to the hydroxyl group of S130. The side chain of W74 is also buried away from the solvent in a hydrophobic pocket containing L58 and V62. The combination of these interactions orientates the catalytic loop (N-terminal to helix 4) correctly with respect to forming a complex with Cdc42. In SopE, Y106 and T110 are in equivalent positions to W74 and E78 in Map; however, they are not involved in inter-helical contacts.

**Figure 4. SopE and the WxxxE bacterial effectors subvert host-cell pathways.** Using the T3SS *Shigella* (A), EPEC/EHEC (B), and *Salmonella* (C) inject the effectors IpgB1/IpgB2, EspT/Map/EspM and SopE/SopE2/SifA/SifB, respectively. Except for SifA and SifB, these effectors activate a cascade of signal transduction pathways starting with activation of Rho GTPases (either Rac1, RhoA or Cdc42), which lead to actin polymerization. Membrane ruffles induced by IpgB1, EspT, SopE and SopE2 allow bacterial engulfment and subsequent internalization into a bacterial containing vacuole (BCV). Invasive EPEC and *Salmonella* remain in the BCV and induce the formation of intracellular actin comets, and *Salmonella*-induced filaments (Sifs), respectively, while *Shigella* escapes to the cytosol where it forms actin tails. In parallel to subversion of actin dynamics IpgB1, IpgB2, SopE and SopE2 induce inflammatory response; EspM, Map, SopE and SopE2 induce tight junction alteration and Map induces mitochondrial dysfunction. SopE and SifA, which is translocated across the SCV via the Spi2 T3SS, play a role in maintaining the SCV. The activity of SifB remains unknown.