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Pathogenicity of *Salmonella*: SopE-mediated membrane ruffling is independent of inositol phosphate signals

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Abstract

Studies [Zhou, D., Chen, L.-M., Hernandez, L., Shears, S.B., and Galán, J.E. (2001) A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host-cell actin cytoskeleton rearrangements and bacterial internalization. Mol. Microbiol. 39, 248–259] with engineered *Salmonella* mutants showed that deletion of *SopE* attenuated the pathogen's ability to deplete host-cell InsP₅ and remodel the cytoskeleton. We pursued these observations: In SopE-transfected host-cells, membrane ruffling was induced, but SopE did not dephosphorylate InsP₅, nor did it recruit PTEN (a cytosolic InsP₅ phosphatase) for this task. However, PTEN strengthened SopE-mediated membrane ruffling. We conclude SopE promotes host-cell InsP₅ hydrolysis only with the assistance of other *Salmonella* proteins. Our demonstration that *Salmonella*-mediated cytoskeletal modifications are independent of inositol phosphates will focus future studies on elucidating alternate pathogenic consequences of InsP₅ metabolism, including ion channel conductance and apoptosis.

Keywords

SopE; InsP₅; Inositolphosphate; Cytoskeleton; Membrane ruffling; *Salmonella*

1. Introduction

Salmonella is an important enteric pathogen of humans and a variety of domestic and wild animals. Infection is initiated in the intestinal tract, and severe disease produces widespread destruction of the intestinal mucosa. *Salmonella* strains can also disseminate from the intestine and produce serious, sometimes fatal infections in a number of systemic organs [1].

When *Salmonella* invades cells, it hijacks a number of the host's cellular signaling systems [2,3]. One dramatic example is the rapid depletion of the cellular InsP₅ pool [4–6]. In fact, no other stimulus is known that can elicit changes in cellular InsP₅ levels at either the rate or the magnitude of that stimulated by *Salmonella*. InsP₅ is a ‘‘hub’’ that serves several cell signaling pathways [7], and the acute changes in its cellular levels may contribute to the pathogenicity of *Salmonella* in three major ways.

First, it has been suggested that the levels of InsP₅ in host cells may affect the development of membrane ruffling and lamellapodial extensions that envelop *Salmonella* and allow the bacteria to invade the cell [3,4]. This is a plausible role for InsP₅ because the dynamics of the actin cytoskeleton are regulated by groups of proteins containing pleckstrin homology (PH)

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domains [8]. PH-domain proteins often bind both inositol phosphates and inositol lipids; the subcellular distribution of these proteins can be affected by changes in the degree of competition between the two classes of ligands [9–11]. Thus, perturbation of the inositol phosphate profile in host-cells may affect the actions of proteins that control the architecture of the actin cytoskeleton [4,12]. Indeed, genetically engineered *Salmonella* mutants that cannot dephosphorylate InsP₅ are also unable to form membrane ruffles and cannot enter cells [4,5].

Second, InsP₅ is a pro-apoptotic antagonist of the Akt protein kinase [9]. Therefore, loss of cellular InsP₅ may underlie the ability of *Salmonella* to activate Akt and promote cell survival during infection [13]. Third, InsP₅ dephosphorylation to Ins(1,4,5,6)P₄ perturbs K⁺ and Cl[−] channel conductances [5,14,15]. This may affect apoptosis [16], and, in the case of intestinal epithelial cells, changes in ionic conductances can augment salt and fluid secretion into the intestinal tract, which may underlie the secretory diarrhoea that the pathogen induces.

It has therefore become important to understand the molecular mechanisms by which *Salmonella* alters inositol phosphate turnover in the host-cell. Two bacterial proteins have appeared to contribute to this phenomenon. First, SopB (also known as SigD), has a phosphatase domain that can directly hydrolyze InsP₅ [4,6]. Furthermore, a *sopB*[−] strain of *S. typhimurium* was found to be less effective than the wild-type strain at dephosphorylating intracellular InsP₅ [4]. In addition, it has been shown that cellular InsP₅ depletion is also attenuated in a *sopE*[−] strain of *S. typhimurium* [4]. Thus, it is believed that SopE in some way facilitates InsP₅ dephosphorylation. The *sopE*[−] strain is also less effective than the wild-type strain at initiating membrane ruffles, which has given further support to the idea that increased InsP₅ metabolism is directly associated with cytoskeletal changes [4].

However, it is difficult to unravel the various molecular mechanisms driving the multitude of different host/pathogen interactions. Since multiple ‘‘attack’’ pathways are invoked, the fact that they might coincide temporally does not necessarily mean that they are directly functionally coupled. Thus, in the current study, we have directly investigated the role of SopE in dephosphorylating InsP₅, and we have also ascertained the extent to which this is linked to cytoskeletal rearrangements. Our approach has been to study these two cellular responses after transfecting host-cells with SopE.

A variety of cell-types have previously been used to study the molecular mechanisms underlying *Salmonella* pathogenicity [17–20]. The choice of cell-type for the current study was driven by an appreciation that the SopE protein might not hydrolyze InsP₅ directly, but might instead recruit a host-cell InsP₅ phosphatase [4,12]. In mammalian cells, there is only one cytosolic candidate with this activity, namely, PTEN [21,22]. To investigate if PTEN is recruited for this role, it was necessary to use a model system in which we could investigate the effects upon InsP₅ levels in both the absence of PTEN, and in the presence of a physiologically relevant level of PTEN. We therefore studied the effects of SopE upon a *PTEN*-null, human glioblastoma, U87MG [23,24], which has been used as a host for a muristerone-inducible *PTEN* gene [23,25].

2. Materials and methods

2.1. Materials

[³H]InsP₅ was prepared as previously described [21]. Non-radioactive Ins(1,3,4,5,6)P₅ was purchased from CellSignals (Columbus OH). Dr. Mike Myers, Cold Spring Harbor Laboratory, NY, supplied the U87MG cells that were used as hosts for the *PTEN* gene under the control of a muristerone promoter [23]. Gene expression was induced by adding 0.5 μM muristerone to the culture medium for 48 h. U87MG cells were cultured in 60 mm dishes in DMEM, supplemented with 10% FBS, 1% penicillin/streptomycin and 1% Na pyruvate. Recombinant

GST-SopB and GST-SopE were kindly provided by Drs. Daoguo Zhou and Jorge Galán, Yale School of Medicine, New Haven, CT. Dr. Galán also kindly provided the SopE_{78–240} construct in a pSG5 vector, and the SopB construct in a pSB965 vector. Cells were transfected using LipofectAMINE 2000 Reagent (GibcoBRL) in accordance with the manufacturer's instructions.

2.2. Inositol phosphate assays

The U87MG cells were radiolabeled for 3 days with 25 $\mu\text{Ci/ml}$ [^3H]inositol (American Radiolabeled Chemicals, St. Louis, MO). At the appropriate times, the media was aspirated and the inositol phosphates were then extracted from the cells using perchloric acid as previously described [26]. The acid-soluble extracts were neutralized with potassium carbonate, and the inositol phosphates were resolved by anion-exchange HPLC as previously described, using phosphate-based elution systems and a SynChropak Q100 SAX column [27, 28]. The radioactivity was assessed either "on-line" using a Flo-1 counter (Packard Instruments), whereupon data were processed using Flo-1 for Windows (v 3.61) and then exported into SigmaPlot (v 8.0) as an ASCII file. In some other experiments, 1 ml fractions were collected from the HPLC eluate. Levels of inositol phosphates (D.P.M.) in each sample were normalized to the level of inositol lipids (see below). For the analysis of [^3H]InsP₅ metabolism in vitro, assays were quenched with perchloric acid, neutralized, and chromatographed on gravityfed anion-exchange columns [26].

2.3. Inositol lipid assays

For some experiments, levels of PtdIns(4,5)P₂ and PtdIns(3,4,5)P₃ were determined as follows: The medium was aspirated from the [^3H]inositol-labeled cells (see above), which were then quenched with 2 ml 1 M HCl. Then, 10 μl of a phosphoinositide mixture (25 mg/ml in 1:1 (v/v) chloroform/methanol; Sigma) was added, and the [^3H]inositol lipids were extracted [29], deacylated [30], and chromatographed on a 250 $\times$ 4.6 mm Partisphere SAX HPLC column [31]. [^3H]GroPtd-Ins(4,5)P₂ standards were generated from [^3H]PtdIns(4,5)P₂ (Perkin-Elmer, Boston, MA); the [^3H]GroPtdIns(3,4,5)P₃ peak was identified from its known elution position [31] and by virtue of its levels increasing 45-fold as a consequence of cell activation for 5 min with 30 ng/ml of PDGF-BB (R&D system, MN) (data not shown). In other experiments, the perchloric acid-insoluble extracts from [^3H]inositol-labeled cells (obtained as described above) were digested overnight at 4 $^{\circ}\text{C}$ with 1 ml of 100 mM NaOH plus Triton (0.1 %) to solubilize all of the inositol lipids (for normalization of the inositol phosphate data, see above).

2.4. Western blot analysis

Cells were lysed with M-PER Mammalian Protein Extraction Reagent (Pierce, IL) supplemented with protease inhibitor cocktail (Roche Diagnostics Corp.) and phosphatase inhibitor mixture (Calbiochem). Lysates were cleared by centrifugation at 4 $^{\circ}\text{C}$ and protein concentration was quantified by using the Bio-Rad protein assay (Bio-Rad Laboratories). Equal amounts of protein (40 μg) were resolved on Nu-PAGE 4–12% pre-cast gel (NOVEX, San Diego, CA) which were subsequently transferred to PVDF membranes. PTEN was detected by using anti-Human PTEN monoclonal antibody (Cascade, MA, 1:2000 dilution). The HRP-linked secondary antibody (Cell Signaling, MA) was used at 1:7000 dilution. Blots were then stripped using 100 mM 2-mercaptoethanol, 2% SDS and 62.5 mM Tris-HCl (pH 6.8). The membrane was then probed with anti-human GAPDH antibody (Ambion TX, 1:14000 dilution) followed by secondary antibody as described above. Blots were developed using Western blot Chemiluminescence Reagent Plus (NEN Life Science Products).

2.5. Confocal immunofluorescence microscopy

All incubation and wash buffers contained phosphate-buffered saline at pH 7.4 (PBS). Cells, grown to subconfluence on coverslips, were washed and fixed in PBS containing 3% paraformaldehyde for 15 min at 4 °C. Cells were washed three times, permeabilized with 1% Triton X-100 for 5 min at room temperature, washed three times, blocked with 1% bovine serum albumin for 30 min at room temperature, and washed three times. For analysis of the membrane ruffling, cells were incubated with Texas Red-X phalloidin (Molecular Probes, cat No. T-7471) at a 1:20 dilution for 20 min at room temperature, followed by three more washes. The Prolong Antifade kit (Molecular Probes, cat No. P-7481) was used to extend the useful lives of the fluorescent probes. Images were obtained using a laser scanning confocal microscope (LSM 410, Carl Zeiss, Inc., Thornwood, NY) mounted on an inverted microscope (Axiovert 135, Zeiss). The objective lens was a C-Apo 40 × 1.2 numerical aperture water immersion lens (Zeiss). At a pinhole setting of 16, a z resolution of 1.1 μm was obtained. For the green fluorescent protein and actin images, the GFP and Texas- Red (representing actin) were excited independently with the 488- and 568-nm (respectively) laser lines of the attached Ar-Kr laser (Melles Griot, Carlsbad, CA). GFP fluorescence was collected with a 515–540-nm band pass filter and Texas Red-X fluorescence with a 610-nm long pass filter. Representative cells are shown in the figures. All images are projections of z-stacks with the exception of the GFP image in Fig. 5, which is a single confocal slice. A smoothing filter was applied to all GFP images. Contrast enhancement was performed separately on all images so that they all would have approximately the same background and the same level of saturation. All image processing was done using LSM Image Examiner 3.5 for Windows (Zeiss). Representative cells are shown in the figures.

3. Results and discussion

3.1. Comparison of the InsP₅ phosphatase activities of recombinant SopE and SopB

It has previously been shown that a *sopE*[−] strain of *S. typhimurium* was less effective than the wild-type strain at both promoting the formation of membrane ruffles and dephosphorylating host-cell InsP₅ [4]. This naturally led to the proposal that InsP₅ metabolism and membrane ruffling were functionally connected, and that SopE contributed to both phenomena [4]. The SopE protein has no apparent phosphatase consensus sequence, but other precedents argue that, by itself, lack of such sequence homology does not exclude the protein having a phosphatase function. For example, the amino-acid sequence of SopE does not reveal the presence of the Dbl homology and PH domains that were thought essential for GEF activity, yet SopE can act as a GEF [18,32]. We therefore examined whether recombinant SopE protein could dephosphorylate InsP₅ in vitro.

The positive control for this experiment was recombinant SopB, which has previously been shown to hydrolyze InsP₅ in vitro [4,6]. SopB and SopE were separately incubated with 10 μM [³H]-labeled InsP₅ for various times, and then the reactions were analyzed by ion-exchange chromatography (Fig. 1). A “batch” elution protocol was employed to separate InsP₄ product from the InsP₅ substrate; a slight (2.2%) spillover of the InsP₅ into the InsP₄ fraction was observed in zero time assays (Fig. 1). Exactly the same spillover was observed in assays containing 1.1 mg/ml SopE, indicating that there was no InsP₅ metabolism (Fig. 1). In contrast, up to 15% of added InsP₅ was dephosphorylated to InsP₄ in assays that contained SopB, even though it was added at 10-fold lower concentrations than SopE (Fig. 1). These data confirm that SopE has no detectable InsP₅ phosphatase activity in vitro.

3.2. The effects of SopE upon InsP₅ turnover and membrane ruffling in PTEN-null glioblastomas

Although recombinant SopE did not dephosphorylate InsP₅ in vitro (see above), it was still important to determine whether SopE might have this function in vivo (see Section 1). We therefore measured the effects upon InsP₅ levels in a mammalian host-cell following transfection with SopE. We also wanted to explore the possibility that SopE might hijack the host-cell's sole cytosolic InsP₅ phosphatase (i.e. PTEN; [21,22]), therefore we employed a human U87MG glioblastoma cell line that is *PTEN*-null [23,24].

We transiently transfected the U87MG *PTEN*-null cells with cDNA encoding an N-terminally truncated SopE_{78–240}. This protein has enhanced stability in mammalian cells because it lacks the lysosomal targeting signal which normally ensures its rapid degradation, but in all other aspects the truncated protein is fully functional [33]. Co-transfection with cDNA for GFP enabled us to identify the cells that were successfully transfected (Fig. 2); transfection efficiency was 50–70%. The degree of membrane ruffling was assessed by confocal microscopy of fixed cells that were labeled for filamentous actin with Phalloidin conjugated to Texas Red-X (Fig. 2). One indication that SopE has a significant effect upon cellular architecture is evidenced by the less elongated, more rounded structure of the transfected cells (Fig. 2). Most of the SopE-transfected cells typically displayed discrete patches of polymerized actin at the plasma membrane (highlighted with arrows in Fig. 2).

We also studied the effects of SopE upon InsP₅ turnover in cells that had been radio-labeled with [³H]inositol. After 3 days of labelling, cells were transfected with SopE, and then the inositol phosphate levels were analyzed by HPLC. Despite the fact that, 24 h after transfection, there was a considerable activation of membrane ruffling (Fig. 2), we found no effect of SopE upon levels of InsP₅ or any other inositol phosphate (Fig. 3A and B). We also assayed InsP₅ levels in cells 6 and 12 h after transfection, even though SopE expression would be low or non-existent at those times [18]. Again, no effect upon InsP₅ levels was observed (Fig. 3C). As a positive control, we also transfected cells with SopB for 24 h. In three independent experiments, the levels of InsP₅ decreased by 25% ($P < 0.002$; Fig. 3C). The internal control for these experiments was InsP₆, the levels of which were unaffected by SopB ([³H]InsP₆ levels in control cells = 121 ± 1 D.P.M./D.P.M. lipids $\times 10^4$; [³H]InsP₆ levels in SopB-transfected cells = 114 ± 2 D.P.M./D.P.M. lipids $\times 10^4$). There was no effect of SopB transfection upon InsP₅ levels after 12 h (data not shown), consistent with insufficient SopB being expressed after such a short time. Our data are the first to show that the induction of membrane ruffles by SopE is functionally independent of InsP₅ dephosphorylation.

SopE has also been suggested to activate phospholipase C [4,12]. However, we found no evidence for this, since levels of InsP₁, InsP₂ and InsP₃ were unaffected by SopE transfection (Fig. 3 and data not shown).

3.3. The effect of PTEN upon InsP₅ turnover and membrane ruffling following SopE transfection of glioblastomas

Earlier work with a *sopE*[−] strain of *S. typhimurium* raised the possibility that SopE might recruit a host-cell InsP₅ 3-phosphatase [4]. PTEN is the only known cytosolic candidate that has this activity [21], but that was absent from the cells used in the experiments described above. Therefore, we next investigated if SopE would affect InsP₅ levels when *PTEN* expression was induced by treating the U87MG cells with 0.5 μ M muristerone for 48 h (Fig. 4). Previous data have shown that this level of PTEN expression is physiologically relevant [23,25]. The induction of PTEN brought about the expected decrease in cellular PtdIns(3,4,5)P₃ content (see Section 2): the level of [³H]PtdIns(3,4,5)P₃ was 784 ± 98 dpm/dish ($n = 3$) in vehicle-treated PTEN-null cells. The corresponding value was 166 ± 40 dpm/dish after our standard

muristerone treatment to induce PTEN. For an internal control, the levels of [^3H]PtdIns(4,5) P_2 were also recorded. These were not significantly altered by muristerone treatment (PTEN-null = 115620 ± 8988 dpm [^3H]PtdIns(4,5) P_2 /dish; PTEN-expressing = 125654 ± 3264 dpm [^3H]PtdIns(4,5) P_2 /dish).

We found that InsP₅ levels in the PTEN-expressing cells did not decrease after SopE transfection (Fig. 5). Clearly, SopE does not activate the InsP₅ 3-phosphatase activity of PTEN. Another finding in these experiments was that the SopE-induced membrane ruffling in the PTEN-expressing cells (Fig. 5) tended to extend over a greater surface of the cell membrane, as compared to the more spatially restricted ruffling that was observed in the absence of PTEN (Fig. 3). We have quantified this phenomenon by counting the number of SopE-transfected cells in which membrane ruffling was confined to less than 33% of the plasma membrane: This occurred in 59% of 240 PTEN-null cells but only in 20% of 270 PTEN-expressing cells. This result of this comparison is somewhat counter-intuitive, given that PTEN expression, through its ability to lower PtdIns(3,4,5) P_3 levels, might generally be anticipated to inhibit membrane ruffling [34,35]. On the other hand, the extensive membrane ruffling that we observed following transient transfection of SopE in PTEN-expressing cells (Fig. 5) recapitulates observations made in previous studies [4,18] in which SopE was transfected into cells with a normal complement of PTEN. It could be an important future direction to determine how this apparent interaction between SopE and PTEN influences the nature of the membrane ruffling response. Perhaps some independent effects of the non-catalytic C2 domain of PTEN [36] can affect SopE function. Additionally, PTEN can modulate the expression of many different proteins, notably including Rac [37], through which SopE exerts its effects upon the cytoskeleton [18].

3.4. Concluding comments

Salmonella utilizes a very sophisticated arsenal of virulence proteins to hijack the host's signal transduction systems, so as to invade and proliferate within host-cells. One particularly striking aspect of this pathogenicity is an ability to deplete the cellular pool of InsP₅ [4–6]; no other stimulus is known that can elicit changes in cellular InsP₅ levels at either the rate or the magnitude of that stimulated by *Salmonella*. Previous studies [4] have shown that SopE plays some role in depleting InsP₅ levels. In the current study, we have come to two new important conclusions concerning the action of SopE: First, InsP₅ dephosphorylation within the host-cell is not promoted by SopE itself, nor is it the result of an interaction of SopE with host proteins. It now seems likely that the contribution that SopE makes to accelerating InsP₅ depletion when the entire bacterium invades cells [4], requires an interaction of SopE with other *Salmonella* virulence proteins.

The second major conclusion to arise from this study is that SopE-dependent remodeling of the actin cytoskeleton occurs independently of changes in inositol phosphate turnover. This is the first time that these two effects of SopE have been functionally separated. This result has wider ramifications; it indicates that the host-cell cytoskeletal rearrangements and InsP₅ depletion caused by another *Salmonella* virulence protein, SopB [4], are also not directly functionally linked. Perhaps actin remodeling by *Salmonella* is served by manipulating the turnover of inositol lipids [17,38], rather than the inositol phosphates.

Our work should serve to focus greater attention on alternate biological consequences that might arise when *Salmonella* perturbs inositol phosphate metabolism. For example, InsP₅ antagonizes the Akt protein kinase [9]. Therefore, loss of cellular InsP₅ may underlie the ability of *Salmonella* to activate Akt and promote cell survival during infection [13]. Dephosphorylation of InsP₅ to Ins(1,4,5,6) P_4 during *Salmonella* invasion also manipulates ion channel activities in the plasma membrane [5,6,14,15]. It is possible that this may also serve to inhibit host-cell apoptosis [16]. Additionally, changes in ion channel conductance can

accelerate salt and fluid secretion from intestinal epithelial cells [14]. Induction of secretory diarrhoea helps *Salmonella* to leave its current host, and to facilitate the location and colonization of new organisms.

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References

1. Guiney DG, Lesnick M. Targeting of the actin cytoskeleton during infection by *Salmonella* strains. *Clin Immunol* 2005;114:248–255. [PubMed: 15721835]
2. Galán JE, Zhou D. Striking a balance: modulation of the actin cytoskeleton by *Salmonella*. *Proc Natl Acad Sci USA* 2000;97:8754–8761. [PubMed: 10922031]
3. Patel JC, Galán JE. Manipulation of the host actin cytoskeleton by *Salmonella* – all in the name of entry. *Curr Opin Microbiol* 2005;8:10–15. [PubMed: 15694851]
4. Zhou D, Chen LM, Hernandez L, Shears SB, Galán JE. A *Salmonella* inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host-cell actin cytoskeleton rearrangements and bacterial internalization. *Mol Microbiol* 2001;39:248–259. [PubMed: 11136447]
5. Eckmann L, Rudolf MT, Ptasznik A, Schultz C, Jiang T, Wolfson N, Tsien R, Fierer J, Shears SB, Kagno MF, Traynor-Kaplan A. d-myo-inositol 1,4,5,6-tetrakisphosphate produced in human intestinal epithelial cells in response to *Salmonella* invasion inhibits phosphoinositide 3-kinase signaling pathways. *Proc Natl Acad Sci USA* 1997;94:14456–14460. [PubMed: 9405634]
6. Norris FA, Wilson MP, Wallis TS, Galyov EE, Majerus PW. SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. *Proc Natl Acad Sci USA* 1998;95:14057–14059. [PubMed: 9826652]
7. Shears, S.B. (2003) Ins(1,3,4,5,6)P5: A signal transduction hub in: *The Handbook of Cell Signaling* (Bradshaw, R. and Dennis, E., Eds.), Academic Press, San Diego.
8. Lemmon MA, Ferguson KM, Abrams CS. Pleckstrin homology domains and the cytoskeleton. *FEBS Lett* 2002;513:71–76. [PubMed: 11911883]
9. Piccolo E, Vignati S, Maffucci T, Innominato PF, Riley AM, Potter BV, Pandol PP, Broggini M, Iacobelli S, Innocenti P, Falasca M. Inositol pentakisphosphate promotes apoptosis through the PI 3-K/Akt pathway. *Oncogene* 2004;23:1754–1765. [PubMed: 14755253]
10. Komander D, Fairservice A, Deak M, Kular GS, Prescott AR, Peter DC, Safrany ST, Alessi DR, van Aalten DM. Structural insights into the regulation of PDK1 by phosphoinositides and inositol phosphates. *EMBO J* 2004;23:3918–3928. [PubMed: 15457207]
11. Kavran, Jm; Klein, DE.; Lee, A.; Falasca, M.; Isako, SJ.; Skolnik, EY.; Lemmon, MA. Specificity and promiscuity in phosphoinositide binding by pleckstrin homology domains. *J Biol Chem* 1998;273:30497–30508. [PubMed: 9804818]
12. Pizarro-Cerda J, Cossart P. Subversion of phosphoinositide metabolism by intracellular bacterial pathogens. *Nat Cell Biol* 2004;6:1026–1033. [PubMed: 15516995]
13. Knodler LA, Finlay BB, Steele-Mortimer O. The *Salmonella* effector protein SopB protects epithelial cells from apoptosis by sustained activation of Akt. *J Biol Chem* 2005;280:9058–9064. [PubMed: 15642738]
14. Feng Y, Wente SR, Majerus PW. Overexpression of the inositol phosphatase SopB in human 293 cells stimulates cellular chloride influx and inhibits nuclear mRNA export. *Proc Natl Acad Sci USA* 2001;98:875–879. [PubMed: 11158563]
15. Bertelsen LS, Paesold G, Marcus SL, Finlay BB, Eckmann L, Barrett KE. Modulation of chloride secretory responses and barrier function of intestinal epithelial cells by the *Salmonella* effector protein SigD. *Am J Physiol Cell Physiol* 2004;287:C939–C948. [PubMed: 15175224]
16. Bortner CD, Cidlowski JA. The role of apoptotic volume decrease and ionic homeostasis in the activation and repression of apoptosis. *Pflugers Arch* 2004;448:313–318. [PubMed: 15107996]

17. Terebiznik MR, Vieira OV, Marcus SL, Slade A, Yip CM, Trimble WS, Meyer T, Finlay BB, Grinstein S. Elimination of host cell PtdIns(4,5)P₂ by bacterial SigD promotes membrane fission during invasion by Salmonella. *Nat Cell Biol* 2002;4:766–773. [PubMed: 12360287]
18. 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;93:815–826. [PubMed: 9630225]
19. Stender S, Friebe A, Linder S, Rohde M, Mirol S, Hardt WD. Identification of SopE2 from *Salmonella typhimurium*, a conserved guanine nucleotide exchange factor for Cdc42 of the host cell. *Mol Microbiol* 2000;36:1206–1221. [PubMed: 10931274]
20. Fu X, Galán JE. A Salmonella protein antagonizes Rac-1 and Cdc-42 to mediate host-cell recovery after bacterial invasion. *Nature* 1999;401:293–297. [PubMed: 10499590]
21. Caffrey JJ, Darden T, Wenk MR, Shears SB. Expanding coincident signaling by PTEN through its inositol 1,3,4,5,6-Pentakisphosphate 3-phosphatase activity. *FEBS Lett* 2001;499:6–10. [PubMed: 11418101]
22. Ali N, Craxton A, Shears SB. Hepatic ins(1,3,4,5)P₄ 3-phosphatase is compartmentalized inside endoplasmic reticulum. *J Biol Chem* 1993;268:6161–6167. [PubMed: 8384201]
23. Stolarov J, Chang K, Reiner A, Rodgers L, Hannon GJ, Wigler MH, Mittal V. Design of a retroviral-mediated ecdysone-inducible system and its application to the expression profiling of the PTEN tumor suppressor. *Proc Natl Acad Sci USA* 2001;98:13043–13048. [PubMed: 11687610]
24. Li DM, Sun H. PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G₁ cell cycle arrest in human glioblastoma cells. *Proc Natl Acad Sci USA* 1998;95:15406–15411. [PubMed: 9860981]
25. Deleu S, Choi K, Pesesse X, Cho J, Sulis ML, Parsons R, Shears SB. Physiological levels of PTEN control the size of the cellular Ins(1,3,4,5,6)P(5) pool. *Cell Signal* 2006;18:488–498. [PubMed: 15979280]
26. Shears, S.B. (1997) Measurement of inositol phosphate turnover in intact cells and cell-free systems in: *Signalling by Inositides: A Practical Approach* (Shears, S.B., Ed.), pp. 33–52, Oxford University Press, Oxford, UK.
27. Balla T, Sim SS, Iida T, Choi KY, Catt KJJ, Rhee SG. Agonist-induced calcium signalling is impaired in fibroblasts overproducing inositol 1,3,4,5-tetrakisphosphate. *J Biol Chem* 1991;266:24179–24726.
28. Saiardi A, Caffrey JJ, Snyder SH, Shears SB. Inositol polyphosphate multikinase (ArgRIII) determines nuclear mRNA export in *Saccharomyces cerevisiae*. *FEBS Lett* 2000;468:28–32. [PubMed: 10683435]
29. Jackson, T.R., Stephens, L.R. and Hawkins, P.T. (1992) Receptor specificity of growth factor stimulated synthesis of 3-phosphorylated inositol lipids in swiss 3t3 cells. *J. Biol. Chem.* 267, 16627–16636. 1714
30. Kirk, C.J., Morris, A.J. and Shears, S.B. (1990) Inositol phosphate second messengers in: *Peptide Hormone Action: A practical Approach* (Siddle, K. and Hutton, J.C., Eds.), pp. 151–184, Oxford University Press, New York.
31. Auger KR, Serunian LA, Solto SP, Libby P, Cantley LC. PDGF-dependent tyrosine phosphorylation stimulates production of novel polyphosphoinositides in intact cells. *Cell* 1989;57:167–175. [PubMed: 2467744]
32. Buchwald G, Friebe A, Galán JE, Hardt WD, Wittinghofer A, Scheffzek K. Structural basis for the reversible activation of a Rho protein by the bacterial toxin SopE. *EMBO J* 2002;21:3286–3295. [PubMed: 12093730]
33. Kubori T, Galán JE. Temporal regulation of salmonella virulence effector function by proteasome-dependent protein degradation. *Cell* 2003;115:333–342. [PubMed: 14636560]
34. Lim MA, Yang L, Zheng Y, Wu H, Dong LQ, Liu F. Roles of PDK-1 and PKN in regulating cell migration and cortical actin formation of PTEN-knockout cells. *Oncogene* 2004;23:9348–9358. [PubMed: 15531926]
35. Vollenweider P, Clodi M, Martin SS, Imamura T, Kavanaugh WM, Olefsky JM. An SH2 domain-containing 5' inositolphosphatase inhibits insulin-induced GLUT4 translocation and growth factor-induced actin filament rearrangement. *Mol Cell Biol* 1999;19:1081–1091. [PubMed: 9891043]

36. Raftopoulou M, Etienne-Manneville S, Self A, Nicholls S, Hall A. Regulation of cell migration by the C2 domain of the tumor suppressor PTEN. *Science* 2004;303:1179–1181. [PubMed: 14976311]
37. Huang, Y., Wernyj, R.P., Norton, D.D., Precht, P., Seminario, M.C. and Wange, R.L. (2005) Modulation of specific protein expression levels by PTEN: identification of AKAP121, DHFR, G3BP, Rap1, and RCC1 as potential targets of PTEN. *Oncogene*.
38. Hernandez LD, Hueffer K, Wenk MR, Galan JE. Salmonella modulates vesicular traffic by altering phosphoinositide metabolism. *Science* 2004;304:1805–1807. [PubMed: 15205533]

Fig 1.

Reactivity of SopB and SopE towards InsP₅ in vitro. Recombinant GST-SopB (0.12 mg/ml final concentration) or GSTSopE (1.1 mg/ml final concentration) was incubated at 30 °C for 0–40 min in 25 µl of assay buffer containing 50 mM HEPES (pH 7.2), 10 mM DTT, 0.35 mg/ml bovine serum albumin. Assays were quenched and neutralized as described in Section 2 and the InsP₄ product was separated from the InsP₅ substrate by batch ion-exchange chromatography [26]. Data shown are triplicates from a representative experiment that was repeated four times with identical results. In other experiments (not shown) SopE also did not dephosphorylate InsP₅ when the assay buffer was supplemented with 2 mM MgSO₄.

Fig 2.

Transient expression of SopE in PTEN-null glioblastomas induces the formation of discrete patches of membrane ruffling. Human PTENnull U87MG glioblastoma cells were either mock-transfected (“control”, left panel), or SopE-transfected (middle and right panels). After 24 h, cells were labeled with Texas Red-X Phalloidin, to identify actin. The yellow arrows highlight the discrete patches of actin that signify membrane ruffling. In the right-hand panel the green colour shows GFP fluorescence in cells in the same field of view as the middle panel. The white scale bar in the GFP image represents 20 μm .

Fig 3.

SopE transfection of PTEN-null glioblastomas does not affect cellular levels of inositol phosphate signals. Glioblastoma cells were radiolabeled with [^3H]inositol as described in Section 2. Cells were either mock-transfected (“control”) or transfected with either SopB, or SopE and GFP, for either 6, 12 or 24 h, and then cellular inositol phosphate levels were determined by HPLC. Two representative HPLC profiles are shown for cells after 24 h. transfection (Panels A and B). Panel C shows levels of InsP_5 at various times after transfection with SopE; data are means and standard errors from 2–4 experiments at each time point. Panel D shows the levels of InsP_5 , 24 h after transfection with SopB; data are means and standard errors from three experiments. $*P < 0.002$.

Fig 4.

Induction of PTEN expression in the glioblastoma cells PTEN-null U87MG glioblastoma cells were treated with either 0.5 μ M muristerone for 48 h or an appropriate volume of the vehicle control. Cell lysates were prepared and 40 μ g of total protein was resolved on a gel and immunoblotted with antibodies specific for PTEN or GAPDH.

Fig 5.

Inositol phosphates and membrane ruffling in SopE-transfected glioblastoma cells in which PTEN is expressed. Cells in which PTEN expression had been induced by 48 h treatment with 0.5 μ M muristerone (see Fig. 4) were either mock-transfected (“control”) or transfected with SopE and GFP for a further 24 h. Cells were labeled with Texas Red-X Phalloidin, to identify actin. Some muristerone-treated cell cultures were also radiolabeled with [3 H]inositol for 3 days prior to transfection (control or SopE) and then inositol phosphate levels were ascertained by HPLC analysis. The white scale bar in the GFP image represents 20 μ m.