INFECTION AND IMMUNITY, Aug. 2004, p. 4331–4335 Vol. 72, No. 8
0019-9567/04/$08.00+0 DOI: 10.1128/IAI.72.8.4331–4335.2004

MINIREVIEW

Modulation and Utilization of Host Cell Phosphoinositides by *Salmonella* spp.

Dan Drecktrah, Leigh A. Knodler, and Olivia Steele-Mortimer*

Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840

Interactions between microbial pathogens and their hosts can be extremely complex. A good example is the facultative intracellular pathogen *Salmonella enterica*, which causes typhoid fever and gastroenteritis in humans. In order to subvert host cell functions, *Salmonella* uses type III secretion systems (TTSSs) to translocate effector proteins directly into the host cell. *Salmonella* pathogenicity island 1 (SPI1) and SPI2 encode two TTSSs that are required for invasion and intracellular survival, respectively. Although the functions of most TTSS effector proteins have yet to be elucidated, it is clear that these proteins affect a diverse set of eukaryotic processes. Research has predominantly concentrated on identifying the host protein targets of *Salmonella* effectors; however, there is now significant evidence that phosphoinositide signaling pathways are also targeted. The implications of this possibility are considerable since it dramatically increases the number of processes that can be modulated by the pathogen.

PHOSPHOINOSITIDES

Phosphoinositides are the phosphorylated products of phosphatidylinositol, which consists of a *myo*-inositol headgroup connected to a diacylglycerol (DAG) via a phosphodiester linkage (Fig. 1). The free hydroxyl groups at positions 3, 4, and 5 of the inositol ring can be phosphorylated in different combinations, yielding seven different phosphoinositides that are essential components of cellular signaling pathways. Together, these molecules regulate the basic processes which determine cell function and activity, including cell growth and differentiation, actin assembly, cell motility, cell death, membrane trafficking, and glucose transport (9, 21, 49, 58). Even though phosphoinositides are minor membrane lipids, their synthesis and degradation must be exquisitely controlled, both spatially and temporally, and there is an almost bewildering array of lipid kinases, phosphatases, and phospholipases (49).

In essence, phosphoinositides initiate cellular processes by recruiting proteins from the cytosol to specific membrane localizations. Proteins that interact with phosphoinositides contain recognition domains, such as the FYVE, PH (pleckstrin homology), and PX (phox homology) domains (26), that associate with various degrees of affinity and specificity with particular phosphoinositides. It should be emphasized that phosphoinositides also act as precursors for soluble inositol polyphosphates and DAG, which are important second messengers. For example, hydrolysis of phosphatidylinositol-4,5-bisphosphate [$PI(4,5)P_2$ (PIP2)] by phospholipase C yields inositol 1,4,5-triphosphate [$Ins(1,4,5)P_3$], which mediates intracellular calcium release, and DAG, which can activate protein kinase C (1). Given the essential and all-encompassing nature of these processes in cell biology, it is not surprising that phosphoinositides also play vital roles in pathogen-host cell interactions. In this minireview we summarize recent data pertaining to the involvement of phosphoinositides in *Salmonella* invasion, vacuole biogenesis, and signal transduction.

PHOSPHOLIPIDS AND BACTERIAL PATHOGENS

A variety of bacterial pathogens modulate phospholipids during interactions with host cells. As reviewed recently (3), receptor-mediated phagocytosis, which involves phosphoinositide 3-kinase (PI3K), is primarily used by host cells to internalize and kill bacteria, but it can also be used by pathogens as a way to gain entry into cells. For example, internalin B, a protein on the surface of *Listeria monocytogenes*, activates PI3K and can mediate internalization of coated latex beads or noninvasive bacteria (2). Type 1 pilus-mediated invasion of uropathogenic *Escherichia coli* also involves PI3K (28). In contrast, invasion by *Salmonella* spp. and *Shigella flexneri* is PI3K independent, and some pathogens, such as enteropathogenic *E. coli*, may actively prevent phagocytosis by blocking PI3K activation (6). Inactivation of the PI3K pathway can also have significant downstream effects, as illustrated by the prevention of monocyte chemoattractant protein 1 expression in macrophages and T-cell proliferation by *Yersinia enterocolitica* YopH tyrosine phosphatase (39). Bacterial pathogens also secrete phospholipases, which have been reviewed elsewhere, that can have dramatic effects on host cells and are often cytolytic (41).

SALMONELLA TRANSLOCATES AN INOSITOL PHOSPHATASE INTO HOST CELLS

The first indication that *Salmonella* could modulate phosphoinositide pathways in host cells was the observation that D-*myo*-inositol 1,4,5,6-tetrakisphosphate [$Ins(1,4,5,6)P_4$] levels were elevated in epithelial cells infected with *S. enterica* serovar Dublin but not in cells infected with other enteric patho-

* Corresponding author. Mailing address: Rocky Mountain Laboratories, 903 South 4th Street, Hamilton, MT 59840. Phone: (406) 363-9292. Fax: (406) 363-9380. E-mail: omortimer@niaid.nih.gov.

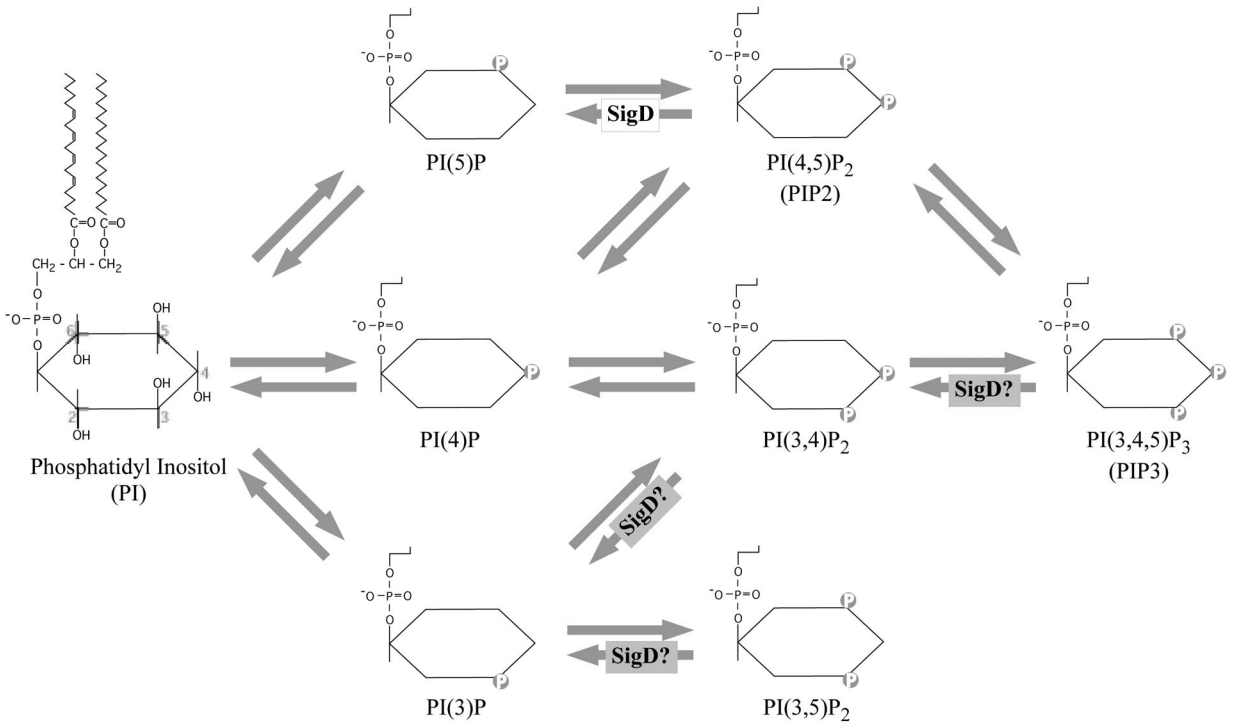

FIG. 1. Phosphoinositide metabolism and involvement of SigD. The actions of phosphoinositide kinases and phosphatases generate seven phosphoinositides from the precursor phosphatidylinositol. Although the *Salmonella* effector protein SigD dephosphorylates several phosphoinositides in vitro (shaded boxes), only PIP2 has been shown to be hydrolyzed in infected mammalian cells (open box) (27, 33, 48, 59).

gens, including *S. flexneri* (10). Subsequent studies revealed that the SPI1 TTSS effectors SigD/SopB (SigD) and SopE are responsible for the accumulation of Ins(1,4,5,6)P_4, albeit via different mechanisms (11, 33, 59).

Initial characterization of SigD revealed the presence of two motifs highly similar to motifs found in inositol polyphosphate 4-phosphatases (Fig. 2). Motif 2 includes a conserved cysteine residue (CKSGKDRTGM), which is essential for the activity of the mammalian enzymes (33). SigD also contains a small region of homology with the mammalian type II inositol 5-phosphatase synaptojanin (27). Although the in vivo substrate(s) is still to be conclusively determined, it has been suggested that SigD hydrolysis of Ins(1,3,4,5,6)P_5, yielding Ins(1,4,5,6)P_4, is crucial for fluid secretion in infected calf intestine loops (33). In vitro SigD can hydrolyze a variety of inositol phosphates and phosphoinositides, including PI(3,4,5)P_3, PI(3,4)P_2, and PI(3,5)P_2 (Fig. 1). This activity is dependent on the conserved cysteine residue in motif 2 and at least two lysine residues in the synaptojanin domain (27, 33). A homologous protein, IpgD, is found in *Shigella* and contains the same conserved motifs (27, 33). While it is unclear whether these homologs have the same in vivo function, they appear to have different substrate specificities in vitro, and *S. flexneri* infection does not increase Ins(1,4,5,6)P_4 levels (27, 31–33). To date, SigD has been shown to affect a number of host cell processes, and, given that multiple pathways are regulated by phosphoinositides and inositol phosphates, it is almost certainly involved in others.

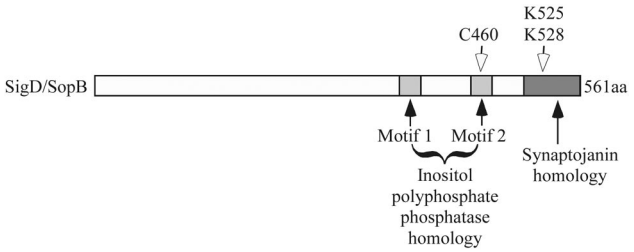

FIG. 2. Schematic representation of SigD/SopB (accession number AAL20023). Regions of homology with human inositol phosphatases and residues essential for phosphatase activity are indicated. aa, amino acids.

SALMONELLA INVASION OF HOST CELLS

Salmonella invasion of nonphagocytic cells, such as epithelial cells, is absolutely dependent on the SPI1 TTSS. Essentially, invasion requires the translocation of a number of SPI1 effectors into the host cell, which leads to rearrangement of the actin cytoskeleton, formation of membrane ruffles, and ultimately internalization of the bacteria. Three SPI1 effectors, SigD, SopE, and SopE2, cooperatively induce the actin rearrangements necessary for invasion. Deletion of any one of these effectors has little effect on invasion, but the triple mutant is noninvasive. This cooperative activity is due to the ability of these effectors to modulate different host cell pathways that ultimately affect actin polymerization. SopE and SopE2 directly activate the Rho-related small GTPases Cdc42 and Rac (14, 20), the major functions of which are the regulation and organization of the actin cytoskeleton. In contrast,

SigD modulates actin polymerization indirectly by altering phospholipid and/or inositol phosphate levels (48, 59). However, further analysis of the roles of SigD and SopE/SopE2 in invasion has revealed some overlap in function. Thus, SigD-mediated entry involves Cdc42, and increases in intracellular Ins(1,4,5,6)P_4 levels can be attributed to SopE in addition to SigD (59). Although the molecular basis of these events has not been characterized, it has been hypothesized that SopE, which has no inositol phosphatase activity, must activate a cellular phosphatase or phospholipases to increase Ins(1,4,5,6)P_4 levels (59). Indeed, both Cdc42 and Rac, which are activated by SopE, can activate phospholipases (19, 22, 55–57). Altogether, these findings indicate that the actin rearrangements necessary for invasion are the result of a complex series of events involving Rho GTPases and phospholipids or inositol phosphates.

Given the essential role of phosphoinositides, particularly PIP2, in actin assembly (58), it is not surprising that PIP2 and PIP3 levels are locally increased in *Salmonella*-induced membrane ruffles (45, 48). Rapid recruitment of the PIP3 interacting protein AKT/PKB to ruffles indicates that there is significant accumulation of this phosphoinositide (45). However, the role of PIP3 in invasion remains unclear since inhibition of PI3K activity by wortmannin (WTM) does not prevent *Salmonella* invasion of epithelial cells (44). PIP2 also accumulates in *Salmonella*-induced ruffles and has an essential, although complex, role in invasion. To speculate on the origin of PIP2 in ruffles, one possibility is that *Salmonella*-induced activation of Rho GTPases activates phosphoinositide 4-phosphate-5-OH kinase, a mediator of Rac-stimulated actin assembly (50). Meanwhile, the PIP2 increase in ruffles is accompanied by a SigD-dependent PIP2 decrease in the invaginating regions (48). SigD-mediated hydrolysis of PIP2 in these regions is a prerequisite for efficient membrane fission and thus initial formation of the *Salmonella*-containing vacuole (SCV) (48). Corroboration of these findings has come from studies with IpgD, the SigD homolog in *S. flexneri*, which mediates hydrolysis of PIP2 that leads to increased membrane blebbing and actin rearrangement (31). Nevertheless, SigD and IpgD are not essential for invasion, and, unlike *Salmonella*, *Shigella* does not cause increases in intracellular levels of Ins(1,4,5,6)P_4. In contrast, IpgD has been shown to increase the intracellular levels of PI(5)P (by hydrolysis of PIP2) and PIP3 (5, 31). In this respect it is important to remember that these proteins, despite their similarities, are delivered by very different pathogens and with different cohorts. It is likely that other translocated effectors are involved in the final outcome in vivo.

BIOGENESIS OF THE SCV

Characterization of the SCV remains largely incomplete since numerous attempts to purify the vacuole to homogeneity have failed. In spite of this, analysis of the distribution of specific proteins in *Salmonella*-infected cells has yielded a basic characterization of SCV biogenesis (24) which indicates that certain phosphoinositides are involved. Newly formed SCVs are characterized by the presence of the early endosomal proteins EEA1 and Rab5, which are rapidly replaced by lysosomal membrane glycoproteins, such as Lamp1 (46). The monophosphorylated phosphoinositide PI(3)P, not to be confused with PIP3, directs membrane association of EEA1 and Rab5 and is usually restricted to early endosomes and the internal membranes of multivesicular endosomes (17). In *Salmonella*-infected epithelial cells, however, it is also found transiently on membrane ruffles and the SCVs, although its role remains unclear (34, 43). Inhibition of PI3K, and thus PI(3)P production, with WTM causes *Salmonella* to escape from the SCV several hours after internalization, and surprisingly, this increases bacterial replication in epithelial cells (4, 44). Since both EEA1 and a PI(3)P probe, FYVE-green fluorescent protein, remain associated with early SCVs after WTM treatment, PI(3)P apparently still accumulates on the vacuole (34, 44). This raises the possibility that *Salmonella* either activates a WTM-insensitive host PI3K or, alternatively, translocates a WTM-insensitive PI3K of its own.

Although the origin, function, and regulation of PI(3)P on the SCV remain unclear, this molecule is likely to play an important role in vacuole biogenesis since it is a key regulator of endocytic and phagocytic membrane trafficking (12, 16, 25, 35, 54). A large number of proteins possess PI(3)P binding domains (42, 47). Several of these, including EEA1, contain the well-characterized FYVE domain and are involved in endocytic membrane trafficking or receptor signaling from the endosome (18, 37, 47). PX domains, originally identified in two components of the phagocyte oxidase ($p40^{phox}$ and $p47^{phox}$), also bind PI(3)P and are found in a variety of proteins implicated in membrane trafficking, protein sorting, and lipid modification (36). Interestingly, phagocyte oxidase is excluded from the SCV in macrophages, via an SPI2-dependent process (7, 8, 15, 52, 53). Hence, one or more SPI2 effectors may be required for the removal of PI(3)P from the SCV. Characterization of the localization of other PI(3)P-binding proteins in *Salmonella*-infected cells is now essential if we are to determine the role of this phosphoinositide in SCV biogenesis.

Recent studies have revealed that actin accumulates around the SCV several hours postinvasion and is necessary for maintaining SCV integrity (29). Thus, actin and presumably its phosphoinositide regulator PIP2 are implicated in SCV biogenesis, as well as invasion. Two SPI2 effectors, SspH2 and SseI, colocalize with the SCV-associated actin and can interact with the actin-binding proteins profilin and filamin (30), which in turn interact directly with PIP2 (23). SseJ is another SPI2 effector that may modulate phosphoinositide levels in the SCV membrane (38). SseJ has homology to several acyltransferases from *Aeromonas* and *Vibrio* species that belong to the GDSL group of lipolytic enzymes (38, 51). Although SseJ is targeted to the SCV membrane after translocation and the acyltransferase activity is required for SseJ-mediated endosome aggregation, no host substrate has been identified for this effector (13, 38). Thus, since no specific SPI2 effector has been shown to be essential for actin accumulation, the mechanism by which PIP2 is generated remains unclear. One intriguing possibility is that the SPI1 effector SigD, by consuming PIP2, actively prevents actin accumulation on the early SCV. According to this hypothesis SPI1 downregulation would be a prerequisite for increased PIP2 levels and the subsequent SPI2-dependent actin accumulation.

INDUCTION OF SIGNAL TRANSDUCTION IN HOST CELLS

Salmonella induces multiple signal transduction pathways in host cells, some of which involve phosphoinositide or inositol phosphate pathways. Most of the identified signal transduction events require the cooperative activity of multiple *Salmonella* effector proteins (59). The exception is the activation of Akt/PKB in epithelial cells that is dependent on the actions of SigD or the *Shigella* homolog IpgD (5, 45). While the mechanism of Akt activation by *Salmonella* has yet to be elucidated, several interesting details have been revealed.

The canonical mechanism of Akt activation involves agonist-mediated activation of PI3K (40). This leads to the generation of PIP3 and $PI(3,4)P_2$ in the plasma membrane, which promote the translocation of Akt and its kinase PDK1 to the membrane. Akt is subsequently phosphorylated at two sites, Ser473 and Thr308. The active form is then released into the cytosol and nucleus, where it stimulates a number of pathways involved in cell growth, cell survival, and glucose metabolism (40). *Salmonella*-induced activation of Akt deviates from this classical model. In particular, activation is potentiated such that Akt is phosphorylated for at least 2 h postinfection, compared to the 5 to 10 min observed after stimulation by epidermal growth factor (45). Perhaps most interesting is the finding that while Akt phosphorylation is SigD dependent, the initial membrane recruitment of Akt is not SigD dependent (45). This has provided us with a novel means to separate the recruitment and activation of Akt into two distinct steps.

SUMMARY

The importance of phosphoinositides in cell biology has only been recognized in the last decade, largely because of the difficulty of working with these molecules. This difficulty has, to some extent, recently been overcome by the development of new technologies. We now know that phospholipids and their metabolites affect almost every process in the eukaryotic cell, and the mechanisms involved in these processes are gradually being elucidated. It is clear that *Salmonella* is also able to modulate the levels of phosphoinositides, both at the plasma membrane and on the SCV. The significance of this cannot be overemphasized since it provides the pathogen with numerous mechanisms to modulate essential host cell processes, including actin remodeling, signal transduction, and membrane trafficking. Unfortunately, at this point there are more questions than answers. For example, what is the function of Akt activation by SigD? Recent data from our laboratory suggest that this process may prevent or delay apoptosis in infected epithelial cells (Knodler and Steele-Mortimer, unpublished observations). What is clear, as we begin to identify the multiple mechanisms by which *Salmonella* interacts with host cells, is that this remarkable interplay presents us with unique opportunities to decipher complex eukaryotic pathways.

REFERENCES

1. **Berridge, M. J.** 1984. Inositol trisphosphate and diacylglycerol as second messengers. Biochem. J. **220:**345–360.
2. **Braun, L., H. Ohayon, and P. Cossart.** 1998. The InIB protein of *Listeria monocytogenes* is sufficient to promote entry into mammalian cells. Mol. Microbiol. **27:**1077–1087.
3. **Brumell, J. H., and S. Grinstein.** 2003. Role of lipid-mediated signal transduction in bacterial internalization. Cell. Microbiol. **5:**287–297.
4. **Brumell, J. H., P. Tang, M. L. Zaharik, and B. B. Finlay.** 2002. Disruption of the *Salmonella*-containing vacuole leads to increased replication of *Salmonella enterica* serovar Typhimurium in the cytosol of epithelial cells. Infect. Immun. **70:**3264–3270.
5. **Carricaburu, V., K. A. Lamia, E. Lo, L. Favereaux, B. Payrastre, L. C. Cantley, and L. E. Rameh.** 2003. The phosphatidylinositol (PI)-5-phosphate 4-kinase type II enzyme controls insulin signaling by regulating PI-3,4,5-trisphosphate degradation. Proc. Natl. Acad. Sci. USA **100:**9867–9872.
6. **Celli, J., M. Olivier, and B. B. Finlay.** 2001. Enteropathogenic *Escherichia coli* mediates antiphagocytosis through the inhibition of PI 3-kinase-dependent pathways. EMBO J. **20:**1245–1258.
7. **Chakravortty, D., I. Hansen-Wester, and M. Hensel.** 2002. *Salmonella* pathogenicity island 2 mediates protection of intracellular *Salmonella* from reactive nitrogen intermediates. J. Exp. Med. **195:**1155–1166.
8. **Chakravortty, D., and M. Hensel.** 2003. Inducible nitric oxide synthase and control of intracellular bacterial pathogens. Microbes Infect. **5:**621–627.
9. **Czech, M. P.** 2003. Dynamics of phosphoinositides in membrane retrieval and insertion. Annu. Rev. Physiol. **65:**791–815.
10. **Eckmann, L., M. T. Rudolf, A. Ptasznik, C. Schultz, T. Jiang, N. Wolfson, R. Tsien, J. Fierer, S. B. Shears, M. F. Kagnoff, and A. E. Traynor-Kaplan.** 1997. 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 **94:**14456–14460.
11. **Feng, Y., S. R. Wente, and P. W. Majerus.** 2001. Overexpression of the inositol phosphatase SopB in human 293 cells stimulates cellular chloride influx and inhibits nuclear mRNA export. Proc. Natl. Acad. Sci. USA **98:**875–879.
12. **Fratti, R. A., J. M. Backer, J. Gruenberg, S. Corvera, and V. Deretic.** 2001. Role of phosphatidylinositol 3-kinase and Rab5 effectors in phagosomal biogenesis and mycobacterial phagosome maturation arrest. J. Cell Biol. **154:**631–644.
13. **Freeman, J. A., M. E. Ohl, and S. I. Miller.** 2003. The *Salmonella enterica* serovar Typhimurium translocated effectors SseJ and SifB are targeted to the *Salmonella*-containing vacuole. Infect. Immun. **71:**418–427.
14. **Friebel, A., H. Ilchmann, M. Aepfelbacher, K. Ehrbar, W. Machleidt, and W. D. Hardt.** 2001. SopE and SopE2 from *Salmonella typhimurium* activate different sets of RhoGTPases of the host cell. J. Biol. Chem. **276:**34035–34040.
15. **Gallois, A., J. R. Klein, L. A. Allen, B. D. Jones, and W. M. Nauseef.** 2001. *Salmonella* pathogenicity island 2-encoded type III secretion system mediates exclusion of NADPH oxidase assembly from the phagosomal membrane. J. Immunol. **166:**5741–5748.
16. **Gaullier, J. M., D. Gillooly, A. Simonsen, and H. Stenmark.** 1999. Regulation of endocytic membrane traffic by phosphatidylinositol 3-phosphate. Biochem. Soc. Trans. **27:**666–670.
17. **Gillooly, D. J., I. C. Morrow, M. Lindsay, R. Gould, N. J. Bryant, J. M. Gaullier, R. G. Parton, and H. Stenmark.** 2000. Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. EMBO J. **19:**4577–4588.
18. **Gillooly, D. J., A. Simonsen, and H. Stenmark.** 2001. Cellular functions of phosphatidylinositol 3-phosphate and FYVE domain proteins. Biochem. J. **355:**249–258.
19. **Han, J. S., H. C. Kim, J. K. Chung, H. S. Kang, J. Donaldson, and J. K. Koh.** 1998. The potential role for CDC42 protein from rat brain cytosol in phospholipase D activation. Biochem. Mol. Biol. Int. **45:**1089–1103.
20. **Hardt, W. D., L. M. Chen, K. E. Schuebel, X. R. Bustelo, and J. E. Galan.** 1998. *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. Cell **93:**815–826.
21. **Hilgemann, D. W.** 2003. Getting ready for the decade of the lipids. Annu. Rev. Physiol. **65:**697–700.
22. **Illenberger, D., F. Schwald, D. Pimmer, W. Binder, G. Maier, A. Dietrich, and P. Gierschik.** 1998. Stimulation of phospholipase C-beta2 by the Rho GTPases Cdc42Hs and Rac1. EMBO J. **17:**6241–6249.
23. **Janmey, P. A.** 1994. Phosphoinositides and calcium as regulators of cellular actin assembly and disassembly. Annu. Rev. Physiol. **56:**169–191.
24. **Knodler, L. A., and O. Steele-Mortimer.** 2003. Taking possession: biogenesis of the *Salmonella*-containing vacuole. Traffic **4:**587–599.
25. **Lawe, D. C., A. Chawla, E. Merithew, J. Dumas, W. Carrington, K. Fogarty, L. Lifshitz, R. Tuft, D. Lambright, and S. Corvera.** 2002. Sequential roles for phosphatidylinositol 3-phosphate and Rab5 in tethering and fusion of early endosomes via their interaction with EEA1. J. Biol. Chem. **277:**8611–8617.
26. **Lemmon, M. A.** 2003. Phosphoinositide recognition domains. Traffic **4:**201–213.
27. **Marcus, S. L., M. R. Wenk, O. Steele-Mortimer, and B. B. Finlay.** 2001. A synaptojanin-homologous region of *Salmonella typhimurium* SigD is essential for inositol phosphatase activity and Akt activation. FEBS Lett. **494:**201–207.
28. **Martinez, J. J., M. A. Mulvey, J. D. Schilling, J. S. Pinkner, and S. J. Hultgren.** 2000. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. EMBO J. **19:**2803–2812.
29. **Meresse, S., K. E. Unsworth, A. Habermann, G. Griffiths, F. Fang, M. J. Martinez-Lorenzo, S. R. Waterman, J. P. Gorvel, and D. W. Holden.** 2001.

Remodelling of the actin cytoskeleton is essential for replication of intravacuolar *Salmonella.* Cell. Microbiol. **3:**567–577.
30. **Miao, E. A., M. Brittnacher, A. Haraga, R. L. Jeng, M. D. Welch, and S. I. Miller.** 2003. *Salmonella* effectors translocated across the vacuolar membrane interact with the actin cytoskeleton. Mol. Microbiol. **48:**401–415.
31. **Niebuhr, K., S. Giuriato, T. Pedron, D. J. Philpott, F. Gaits, J. Sable, M. P. Sheetz, C. Parsot, P. J. Sansonetti, and B. Payrastre.** 2002. Conversion of PtdIns(4,5)P(2) into PtdIns(5)P by the *S. flexneri* effector IpgD reorganizes host cell morphology. EMBO J. **21:**5069–5078.
32. **Niebuhr, K., N. Jouihri, A. Allaoui, P. Gounon, P. J. Sansonetti, and C. Parsot.** 2000. IpgD, a protein secreted by the type III secretion machinery of *Shigella flexneri*, is chaperoned by IpgE and implicated in entry focus formation. Mol. Microbiol. **38:**8–19.
33. **Norris, F. A., M. P. Wilson, T. S. Wallis, E. E. Galyov, and P. W. Majerus.** 1998. SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. Proc. Natl. Acad. Sci. USA **95:**14057–14059.
34. **Pattni, K., M. Jepson, H. Stenmark, and G. Banting.** 2001. A PtdIns(3)P-specific probe cycles on and off host cell membranes during *Salmonella* invasion of mammalian cells. Curr. Biol. **11:**1636–1642.
35. **Petiot, A., J. Faure, H. Stenmark, and J. Gruenberg.** 2003. PI3P signaling regulates receptor sorting but not transport in the endosomal pathway. J. Cell Biol. **162:**971–979.
36. **Ponting, C. P.** 1996. Novel domains in NADPH oxidase subunits, sorting nexins, and PtdIns 3-kinases: binding partners of SH3 domains? Protein Sci. **5:**2353–2357.
37. **Raiborg, C., T. E. Rusten, and H. Stenmark.** 2003. Protein sorting into multivesicular endosomes. Curr. Opin. Cell Biol. **15:**446–455.
38. **Ruiz-Albert, J., X. J. Yu, C. R. Beuzon, A. N. Blakey, E. E. Galyov, and D. W. Holden.** 2002. Complementary activities of SseJ and SifA regulate dynamics of the *Salmonella typhimurium* vacuolar membrane. Mol. Microbiol. **44:**645–661.
39. **Sauvonnet, N., I. Lambermont, P. van der Bruggen, and G. R. Cornelis.** 2002. YopH prevents monocyte chemoattractant protein 1 expression in macrophages and T-cell proliferation through inactivation of the phosphatidylinositol 3-kinase pathway. Mol. Microbiol. **45:**805–815.
40. **Scheid, M. P., and J. R. Woodgett.** 2003. Unravelling the activation mechanisms of protein kinase B/Akt. FEBS Lett. **546:**108–112.
41. **Schmiel, D. H., and V. L. Miller.** 1999. Bacterial phospholipases and pathogenesis. Microbes Infect. **1:**1103–1112.
42. **Schultz, J., R. R. Copley, T. Doerks, C. P. Ponting, and P. Bork.** 2000. SMART: a web-based tool for the study of genetically mobile domains. Nucleic Acids Res. **28:**231–234.
43. **Scott, C. C., P. Cuellar-Mata, T. Matsuo, H. W. Davidson, and S. Grinstein.** 2002. Role of 3-phosphoinositides in the maturation of *Salmonella*-containing vacuoles within host cells. J. Biol. Chem. **277:**12770–12776.
44. **Steele-Mortimer, O., J. H. Brumell, L. A. Knodler, S. Meresse, A. Lopez, and B. B. Finlay.** 2002. The invasion-associated type III secretion system of *Salmonella enterica* serovar Typhimurium is necessary for intracellular proliferation and vacuole biogenesis in epithelial cells. Cell. Microbiol. **4:**43–54.
45. **Steele-Mortimer, O., L. A. Knodler, S. L. Marcus, M. P. Scheid, B. Goh, C. G. Pfeifer, V. Duronio, and B. B. Finlay.** 2000. Activation of Akt/protein kinase B in epithelial cells by the *Salmonella typhimurium* effector SigD. J. Biol. Chem. **275:**37718–37724.
46. **Steele-Mortimer, O., S. Meresse, J. P. Gorvel, B. H. Toh, and B. B. Finlay.** 1999. Biogenesis of *Salmonella typhimurium*-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway. Cell. Microbiol. **1:**33–49.
47. **Stenmark, H., R. Aasland, and P. C. Driscoll.** 2002. The phosphatidylinositol 3-phosphate-binding FYVE finger. FEBS Lett. **513:**77–84.
48. **Terebiznik, M. R., O. V. Vieira, S. L. Marcus, A. Slade, C. M. Yip, W. S. Trimble, T. Meyer, B. B. Finlay, and S. Grinstein.** 2002. Elimination of host cell PtdIns(4,5)P(2) by bacterial SigD promotes membrane fission during invasion by *Salmonella.* Nat. Cell Biol. **4:**766–773.
49. **Toker, A.** 2002. Phosphoinositides and signal transduction. Cell Mol. Life Sci. **59:**761–779.
50. **Tolias, K. F., J. H. Hartwig, H. Ishihara, Y. Shibasaki, L. C. Cantley, and C. L. Carpenter.** 2000. Type Ialpha phosphatidylinositol-4-phosphate 5-kinase mediates Rac-dependent actin assembly. Curr. Biol. **10:**153–156.
51. **Upton, C., and J. T. Buckley.** 1995. A new family of lipolytic enzymes? Trends Biochem. Sci. **20:**178–179.
52. **Vazquez-Torres, A., G. Fantuzzi, C. K. Edwards III, C. A. Dinarello, and F. C. Fang.** 2001. Defective localization of the NADPH phagocyte oxidase to *Salmonella*-containing phagosomes in tumor necrosis factor p55 receptor-deficient macrophages. Proc. Natl. Acad. Sci. USA **98:**2561–2565.
53. **Vazquez-Torres, A., Y. Xu, J. Jones-Carson, D. W. Holden, S. M. Lucia, M. C. Dinauer, P. Mastroeni, and F. C. Fang.** 2000. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science **287:**1655–1658.
54. **Vieira, O. V., C. Bucci, R. E. Harrison, W. S. Trimble, L. Lanzetti, J. Gruenberg, A. D. Schreiber, P. D. Stahl, and S. Grinstein.** 2003. Modulation of Rab5 and Rab7 recruitment to phagosomes by phosphatidylinositol 3-kinase. Mol. Cell. Biol. **23:**2501–2514.
55. **Walker, S. J., W. J. Wu, R. A. Cerione, and H. A. Brown.** 2000. Activation of phospholipase D1 by Cdc42 requires the Rho insert region. J. Biol. Chem. **275:**15665–15668.
56. **Woo, C. H., B. C. Kim, K. W. Kim, M. H. Yoo, Y. W. Eom, E. J. Choi, D. S. Na, and J. H. Kim.** 2000. Role of cytosolic phospholipase A(2) as a downstream mediator of Rac in the signaling pathway to JNK stimulation. Biochem. Biophys. Res. Commun. **268:**231–236.
57. **Woo, C. H., Z. W. Lee, B. C. Kim, K. S. Ha, and J. H. Kim.** 2000. Involvement of cytosolic phospholipase A2, and the subsequent release of arachidonic acid, in signalling by rac for the generation of intracellular reactive oxygen species in rat-2 fibroblasts. Biochem. J. **348:**525–530.
58. **Yin, H. L., and P. A. Janmey.** 2003. Phosphoinositide regulation of the actin cytoskeleton. Annu. Rev. Physiol. **65:**761–789.
59. **Zhou, D., L. M. Chen, L. Hernandez, S. B. Shears, and J. E. Galan.** 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.

Editor: J. B. Kaper