PATHOGENIC TRICKERY: DECEPTION OF HOST CELL PROCESSES

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Microbial pathogens cause a spectrum of diseases in humans. Although the disease mechanisms vary considerably, most pathogens have developed virulence factors that interact with host molecules, often usurping normal cellular processes, including cytoskeletal dynamics and vesicle targeting. These virulence factors often mimic host molecules, and mediate events as diverse as bacterial invasion, antiphagocytosis, and intracellular parastism.

PHAGOCYTOSIS
Actin-dependent process, by which cells engulf external particulate material by extension and fusion of pseudopods.

GRAM-POSITIVE BACTERIA
Bacteria with cell walls that retain a basic blue dye during the Gram-stain procedure. These cell walls are relatively thick, consisting of a network of peptidoglycan and lipoteichoic acids.

E-CADHERIN
Calcium-dependent adhesion molecule that mediates homophilic adhesions between epithelial cells.

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Pathogens have developed sophisticated mechanisms to either block or subvert normal host cellular processes, thereby inadvertently contributing to pathogenesis and disease outcome. Over the years, a recurring theme in these host–pathogen interactions has emerged — bacterial mimicry of host cell molecules to subvert cellular functions. For example, bacterial surface proteins can mimic receptor ligands to engage cell surface receptors and their associated signalling pathways to promote bacterial uptake. Moreover, secretion systems have been identified in various bacterial pathogens that function as molecular syringes to inject proteins, called effectors, directly into mammalian cells. Here they can directly intercept specific cellular targets, instead of relying on endocytic processes for delivery. Prime targets for pathogen disruption include the cytoskeleton, signal-transduction pathways and vesicle transport. By altering these cellular processes, pathogens can block PHAGOCYTIC uptake, direct their own uptake into a protected intracellular niche and generally subvert the host cell for their own benefit. Although specific molecular mechanisms differ between organisms, the general concept that pathogens have developed molecular tools that subvert normal cellular events is becoming a common tenet in infectious diseases. Studying these tools also provides many fascinating and useful molecules to dissect host cellular processes.

Mimicry of surface receptor ligands

Mammalian cells interact extensively with each other through cell surface receptors, which, upon ligand binding, transduce signals into a cellular response. They also link the extracellular matrix to intracellular scaffolding structures and signalling pathways, either directly, or indirectly through intermediate molecules[1–3]. Many pathogenic bacteria have evolved mechanisms to engage host cell surface proteins or receptors to initially adhere to, and subsequently invade, host cells (TABLE 1). They often achieve this by mimicking host cell ligands. By binding host receptors, the pathogens rely on the normal cellular processes to invade cells.

Listeria monocytogenes is a GRAM-POSITIVE foodborne opportunistic pathogen that can penetrate into host tissue by invasion and replication within non-phagocytic cells, especially hepatocytes. At least two bacterial surface proteins are essential for invasion by *Listeria*. Internalin, encoded by *inlA*, is covalently linked to the bacterial cell wall[4]. Its predicted structure is reminiscent of other Gram-positive bacterial ligands known to bind extracellular matrix proteins[5]. Internalin interacts with the epithelial cell receptor, E-CADHERIN[6] through its amino-terminal region of leucine-rich repeats (LRR). The amino-terminal region of internalin is necessary and sufficient for *Listeria* attachment to, and invasion of, epithelial cells[7]. A single amino-acid residue in E-cadherin (Pro16) was recently identified as crucial for

Table 1 | Examples of pathogens that engage host cell surface receptors

Bacterium	Bacterial ligand	Natural ligand	Host cell surface receptor	Reference
Listeria monocytogenes	InlA	Human mucosal lymphocyte-1 antigen (HML-1)	E-cadherin	6
	InlB	Hepatocyte growth factor	c-Met	15
		Complement component C1q	C1q	14
	ActA	Heparan sulfate proteoglycans	Heparin sulfate receptor	97
Yersinia enterocolitica	Invasin	Fibronectin	$\alpha_5\beta_1$ integrin	98
	YadA (through extracellular-matrix protein bridge)	Extracellular matrix proteins	Integrins	99
Shigella flexneri	IpaB–IpaC	Fibronectin	$\alpha_5\beta_1$ integrin	57
	IpaB	Hyaluronic acid	CD44	58
Neisseria gonorrhoeae/ N. meningitidis	OpaA	Extracellular matrix proteins Cytokines Growth factors Proteases	Heparan sulfate proteoglycans	100
	OpaA (through vitronectin bridge)	Vitronectin Fibrillin	$\alpha_v\beta_5/\alpha_v\beta_3$ integrins	101
	Opa proteins	CD66 Selectins	CEA (CD66a, CD66c, CD66d)	102 103
	Opc (through vitronectin bridge)	Vitronectin	Endothelial $\alpha_v\beta_3$ integrins	104
	Opc	Extracellular matrix proteins Cytokines	Epithelial heparan sulfate proteoglycans	105
Mycobacterium kansasii	GPI-anchored surface proteins	Complement fragment C3bi Fibrinogen ICAM-1	CR3 ($\alpha_M\beta_2$ integrin; CD11b/CD18)	28
Escherichia coli	FimH adhesin	Heparan sulfate 2B4	CD48	27
	FimH adhesin	CD2	Mannose-containing receptors	106
	PapG	Glycolipids	Globoside	107
	AF/R1 fimbriae	Disaccharides	Sucrase-isomaltase protein (S1) complex	108
	HlyA toxin	ICAM-1 ICAM-2 ICAM-3	$\alpha_L\beta_2$ integrin (CD11a/CD18)	109
Leishmania donovani	GP63	C3bi Fibrinogen ICAM-1	CR3	110
Staphylococcus aureus	Fibronectin-binding protein A and B	Fibronectin	$\alpha_5\beta_1$ integrins	111
Streptococcus pyogenes	Protein F/M (through fibronectin bridge)	Fibronectin	$\alpha_5\beta_1$ integrins	112
Bordetella pertussis	CyaA toxin	Fibrinogen C3bi ICAM-1	CR3 receptor	113
Pasteurella Haemolytica	LktA toxin	ICAM-1 ICAM-2 ICAM-3	$\alpha_L\beta_2$ integrin (CD11a/CD18)	114

CEA, carcinoembryonic antigen; CR3, complement receptor; GPI, glycosylphosphatidylinositol; ICAM, intracellular adhesion molecule.

InlA binding[8,9]. InlA–E-cadherin interactions cause the plasma membrane to surround the invading *Listeria* and eventually engulf the bacterium in a zipper-like process. The invasion process involves both microfilaments and microtubules[10], but the exact mechanism of how InlA–E-cadherin interactions result in bacterial uptake is not known.

Ligand–receptor interactions in other non-phagocytic cell types also results in *Listeria* invasion, but involves different yet related players. InlB has significant amino-acid sequence similarity to InlA, including an amino-terminal LRR region, and is involved in the invasion of some epithelial and endothelial cells, fibroblasts and hepatocytes. Tandem repeats in the carboxyl terminus of InlB anchor it to lipoteichoic acid in the bacterial membrane[11]. The LRR region, which is crucial for bacterial entry, forms a long curved structure that seems well suited for contact with a receptor[12]. Recently, two mammalian receptors for InlB were identified, gC1q-R[13] and Met tyrosine kinase (Met)[14]. gC1q-R, which is expressed on a wide variety of cell types, is the receptor of the globular part of the complement component C1q, but it also has affinity for an array of other ligands[15]. Both C1q and InlB bind to the amino-terminal region of gC1q-R[13]. The receptor tyrosine kinase Met has a cytoplasmic tail with tyrosine kinase activity. Binding of the natural ligand, hepatocyte growth factor (HGF), induces diverse signalling events mediated by phosphatidylinositol 3-kinase (PI3K), leading to cytoskeletal changes, apoptosis, and cell growth and differentiation[16,17]. Likewise, InlB is also a known agonist of PI3K[18], and InlB–Met interactions stimulate the association of tyrosine-phosphorylated proteins with PI3K[14]. In a classical case of convergent evolution, although InlB and HGF do not seem to bind the same sites on Met[14], InlB stimulates a similar cascade of events as the natural ligand, which includes actin cytoskeletal rearrangements and membrane ruffling. So, unlike InlA, which mediates a zipper-like uptake mechanism, InlB regulates a trigger-mediated mechanism that uses host signalling pathways.

Invasion of epithelial cells and M CELLS by *Yersinia* spp. is also due to an interaction between bacterial ligands and mammalian receptors. The invasin protein of *Yersinia pseudotuberculosis* binds through its carboxyl terminus to a subset of β_1 INTEGRINS ($\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$, $\alpha_v\beta_1$)[19]. Although they do not have sequence homology, invasin and fibronectin — the natural ligand — seem to bind to the same site on $\alpha_5\beta_1$ integrin[20]. However, invasin binds with a 100-fold greater affinity than fibronectin[21]. Whereas the initial invasin–integrin interactions mediate bacterial adherence, it is thought that the increased binding affinity is essential for bacterial invasion. Invasin molecules probably interact with each other after integrin binding to cluster the integrin receptors. Such receptor multimerization around the bacterium surface could mediate cytoskeletal rearrangements and zipper the plasma membrane around the adherent *Yersinia*, in a process analogous to *Listeria* invasion.

In response to binding by a natural ligand, the cytoplasmic tail of both integrin receptor subunits regulates receptor function. In the case of invasin binding, residues within the β_1 chain cytoplasmic domain are essential for bacterial uptake[22]. This domain can recruit and interact directly with cytoskeletal proteins such as talin and α-actinin, and kinases such as focal adhesion kinase (FAK) and mitogen-activated protein kinases[3]. Invasin-mediated uptake involves FAK, which might activate downstream signalling mediators, including the focal adhesion protein CAS and a member of the Rho family, Rac1 (REFS 23,24). How these signals are integrated into the cytoskeletal rearrangements promoting invasion remains to be determined.

It is becoming evident that bacterial pathogens can also invade cells through LIPID RAFTS, by targeting receptors that are concentrated in these signalling platforms[25,26]. The first example of such a bacterial–host cell interaction was for *fimH*-expressing *E. coli* and mast cells[27]. FimH is a component of type I fimbriae. Under non-OPSONIC conditions FimH binds to the GPI-ANCHORED molecule CD48, which is associated with CAVEOLAE, and this triggers phagocytosis of the bacterium. *fimH*-expressing bacteria are subsequently enclosed within a protective intracellular vacuole. Lipid rafts have also been implicated in the non-opsonic phagocytosis of *Mycobacterium kansasii* by neutrophils[28]. In this case, CR3, which is normally a receptor for extracellular matrix proteins, can also associate with GPI-anchored proteins in lipid rafts and function as a receptor for *M. kansasii*. Similarly, cholesterol has an essential role in *Mycobacterium bovis* uptake into macrophages[29]. The pathogens uropathogenic *Escherichia coli*[30], *Campylobacter jejuni*[31], *Toxoplasma gondii*[32] and *Plasmodium falciparum*[33] have also been reported to use lipid microdomains for their entry into cells, but details are scant. As vesicles derived from lipid rafts are probably transported independently of other transport pathways and probably do not fuse with endosomes or lysosomes, invasion through lipid rafts represents a prime mechanism for the bacterium to remain in a safe intracellular environment. As such, it is likely that many more bacterial pathogens will be found to target receptors in these signalling platforms.

Coercion of host cell signalling pathways

Whereas pathogens with an intracellular lifestyle have developed many sophisticated strategies to enter and survive within host cells, some bacterial pathogens prevent their own uptake. To achieve this goal, another means of subverting host cell processes is by the injection of bacterial proteins directly into the host cell. Several GRAM-NEGATIVE pathogenic bacteria use specialized secretion systems that are dedicated to the delivery of these bacterial proteins, called effectors[34]. Type III secretion systems span both the bacterial and host cell membranes and are assembled from at least 20 proteins into a structure resembling a 'molecular syringe'. Both invasion and ANTIPHAGOCYTOSIS require the coordinated action of many bacterial effectors. The translocated

M CELL
'Membranous' or 'microfold' cell. Specialized cell of follicle-associated epithelium (FAE) in the gut that internalizes macromolecules and microorganisms and delivers them to the underlying lymphoid tissue.

INTEGRINS
A large family of heterodimeric transmembrane proteins that act as receptors for cell-adhesion molecules.

LIPID RAFTS
Dynamic assemblies of cholesterol and sphingolipids in the plasma membrane.

OPSONIZED
Covered with blood-serum proteins — complement, or immunoglobulin-G antibodies — that enhance uptake by phagocytosis.

GPI ANCHOR
A post-translational modification that anchors proteins to membranes, possibly to domains therein. The anchor is made of one molecule of phosphatidylinositol to which a carbohydrate chain is linked through the C-6 hydroxyl of the inositol, and it is linked to the protein through an ethanolamine phosphate moiety.

CAVEOLA
Flask-shaped, cholesterol-rich invagination of the plasma membrane that might mediate the uptake of some extracellular materials, and is probably involved in cell signalling.

GRAM-NEGATIVE BACTERIA
Bacteria with cell walls that do not retain a basic blue dye during the Gram-stain procedure. These cell walls are composed of two membranes, separated by a thin layer of peptidoglycan. The outer membrane anchors surface lipopolysaccharides.

ANTIPHAGOCYTOSIS
Ability of bacteria to inhibit the cytoskeletal rearrangements at the plasma membrane that would lead to their phagocytosis.

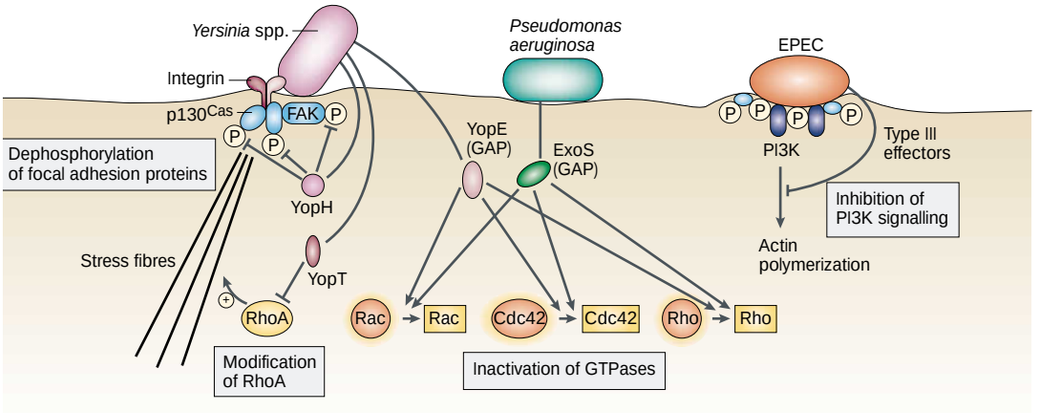

Figure 1 | **Mechanisms through which bacterial pathogens inhibit phagocytosis.** Upon contact, *Yersinia* injects Yop effectors. The phosphotyrosine phosphatase YopH dephosphorylates proteins from the focal adhesion complexes to interrupt integrin-mediated signalling. YopT deactivates RhoA through an unknown mechanism. YopE harbours a GTPase-activating protein (GAP) activity towards Rho, Rac1 and Cdc42. All these effectors contribute to antiphagocytosis by interrupting signalling events that are necessary for cytoskeletal rearrangements. *Pseudomonas aeruginosa* injects through its type III secretion system ExoS, whose GAP activity towards Rho, Rac and Cdc42 is thought to account for antiphagocytosis. Enteropathogenic *Escherichia coli* (EPEC) inhibits phosphatidylinositol 3-kinase (PI3K)-dependent cytoskeletal rearrangements required for its uptake, through the type III translocation of effectors. Orange circles indicate GTP-bound active GTPases, whereas yellow boxes indicate GDP-bound inactive GTPases. FAK, focal adhesion kinase.

effectors typically target biochemical activities of host cell components by functional mimicry.

Phagocytosis and antiphagocytosis both require the manipulation of the actin cytoskeleton. Central to the organization of the actin cytoskeleton are small GTPases of the Rho family — Rho, Rac and Cdc42, which regulate the formation of actin stress fibres and focal contacts, membrane ruffles, and filopodia, respectively[35]. Given the central role that these small GTPases have in the organization of the actin cytoskeleton, it is not surprising that they are directly targeted by many bacterial effectors during both bacterial invasion and antiphagocytosis.

Putting on the brakes: antiphagocytosis. Professional phagocytic cells, such as neutrophils and macrophages, use phagocytosis to internalize and destroy foreign objects, such as pathogens, as well as apoptotic cells. Engagement of phagocytic receptors after particle binding activates cellular pathways that lead to rearrangements of the actin cytoskeleton. Phagocytic pathways are diverse and extremely complex. Opsonin-dependent phagocytosis involves either Fcγ-receptors (Fcγ-R) or complement receptors (CR1, CR3 and CR4) which bind particles that have either IgG or complement bound to their surface, respectively[36]. Although these pathways share common steps, they might also differ depending on the particular receptor engaged. This is exemplified by the role of different Rho-family small GTPases: Rac1 and Cdc42 are involved in Fcγ-R-mediated phagocytosis, whereas RhoA is required for CR3-mediated phagocytosis[37]. Another prominent difference between these two pathways is that protein tyrosine kinases are only required during Fcγ-R-mediated phagocytosis[36]. Functionally, Fcγ-R engagement is accompanied by a respiratory burst and initiation of a proinflammatory cascade, whereas CR3-mediated phagocytosis is not[37].

Microbial pathogens recognize phagocytosis as either an opportunity or an obstacle to their own survival and replication, and respond accordingly. Blocking phagocytosis allows the pathogen to avoid destruction through the DEGRADATIVE ENDOCYTIC PATHWAY and, in some instances, paralyse phagocytic responses and subsequently impair the development of cellular immunity. Several bacterial pathogens have antiphagocytic capabilities (FIG. 1)[38–40,47–49]. One of the better studied antiphagocytic bacteria is *Yersinia*, subspecies of which cause gastrointestinal symptoms and plague. After bacterial ingestion by the host, pathogenic *Yersinia* must first cross the intestinal barrier by invading intestinal epithelial cells and M cells. But then, it needs to avoid internalization by phagocytic cells to avoid destruction. Both processes (invasion and antiphagocytosis) are mediated by interactions between bacterial ligands and cell surface receptors, ironically with opposite effects. To prevent phagocytosis by macrophages after β_1-integrin engagement, *Yersinia* translocates several effectors into host cells. Type III secretion systems are ideally designed to deliver effectors at the site of bacterial contact, where phagocytosis is initiated, and interrupt phagocytic signalling pathways that are triggered upon receptor engagement (FIG. 1). The bacterial effector YopH is localized to focal complexes[41], where its carboxy-terminal tyrosine phosphatase activity specifically targets and dephosphorylates components of focal contacts involved in bacterial uptake. The amino-terminal domain of YopH, which is involved in host cell substrate recognition and phosphotyrosine binding[42], has a compact structure that does not resemble other phosphotyrosine-binding domains[43]. Host proteins that are dephosphorylated by

DEGRADATIVE ENDOCYTIC PATHWAY
Macromolecules endocytosed at the plasma membrane first arrive in early endosomes, from where they are transported to late endosomes, and finally lysosomes where they are degraded by hydrolases.

YopH include p130^{Cas}, Fyb, SKAP-HOM and Crk[24,44–47]. The details of how such dephosphorylation events mediate the cytoskeletal changes required for inhibition of both integrin-mediated and FcγR-mediated phagocytosis[48] remain to be elucidated. YopE, another *Yersinia* effector, disrupts actin filaments by exerting GAP activity on the Rho GTPases Rac-1 and Cdc42 (REF. 49), which are essential to phagocytosis. YopT also depolymerizes actin filaments, probably by modifying, and thereby inactivating, RhoA[50]. So *Yersinia* uses many effectors with complementary actions to mediate antiphagocytic activity.

Much like *Yersinia*, *Pseudomonas aeruginosa* mediates antiphagocytic effects by using its type III secretion system to deliver at least two effectors, ExoS and ExoT, into host cells (FIG. 1). These effectors have GAP activities that affect RhoA, Rac1 and Cdc42[51,52]. This shows that at least two pathogenic bacteria that cause remarkably different clinical diseases (*P. aeruginosa* affects immunocompromised patients, including those with burns and cystic fibrosis), have evolved similar strategies to target and disrupt essential regulatory proteins of phagocytic signalling processes.

However, bacterial pathogens have a broader repertoire of strategies to block phagocytosis, including targeting of other components of phagocytic signal-transduction cascades. For instance, the extracellular diarrhoeal pathogen enteropathogenic *Escherichia coli* (EPEC) prevents its uptake by macrophages by inhibiting the PI3K-dependent phagocytic signals that are essential for the polymerization of F-actin (FIG. 1)[40,53]. The EPEC antiphagocytic activity is mediated by delivery of still unknown effectors through type III secretion systems. Type III secretion systems are not the sole translocation system involved in mediating antiphagocytosis. *Helicobacter pylori* prevents its uptake by professional phagocytes using a type IV secretion system[39,54], which is functionally analogous, but mechanistically unrelated.

Some pathogens can paralyse the host defence mechanism of phagocytosis to avoid clearance. The ability to disrupt the actin cytoskeleton and its associated signals is mediated by localized delivery of potent bacterial effectors into phagocytic cells. Although the outcome is the same, the exact mechanisms exploited by these different pathogens seem to be distinct, but with some overlap. This most probably reflects the diversity of the receptor-mediated signalling pathways triggered upon initial pathogen contact with the phagocytic cell.

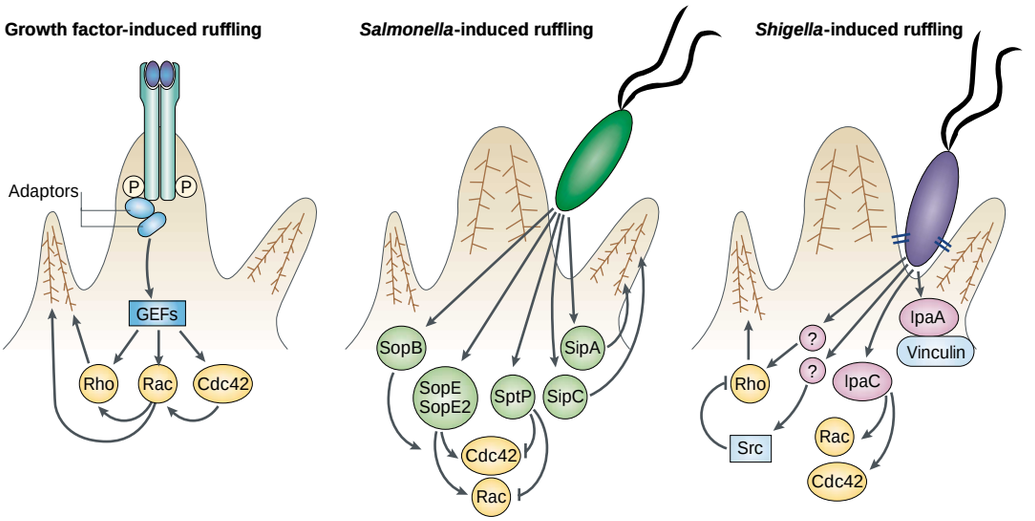

Figure 2 | **A common requirement for the Rho subfamily of GTPases in *Salmonella*-, *Shigella*- and growth factor-induced membrane ruffling.** Engagement of receptor tyrosine kinases by ligands, such as growth factors, initiates a spectrum of cellular responses, including the rapid reorganization of the actin microfilament network. This results in membrane ruffling and the formation of lamellipodia at the cell surface. Signalling from the activated receptor to the actin cytoskeleton is through many intermediate molecules, including adaptor proteins and the Rho subfamily of GTP-binding proteins comprising Rac, Rho and Cdc42. The specific contributions of individual Rho GTPases and hierachy of their activation seem to be specific to cell type and stimulus. *Salmonella* and *Shigella* translocate several effectors directly into the host cell through their type III secretion systems to target both Rho GTPases and the actin cytoskeleton directly. The coordinate action of these effectors promotes membrane ruffling and bacterial invasion. Although *Salmonella* and *Shigella* induce a phenotypically similar response, different Rho proteins are involved in these processes. *Shigella* might initially adhere to host cells by IpaB–CD44 and IpaB/C–$\alpha_5\beta_1$ integrin interactions. Injection of IpaB and IpaC is required for actin polymerization. IpaC also indirectly activates Rac and Cdc42, which results in the formation of lamellipodia. Activation of Rho is also required for *Shigella* entry, but the mechanism is not known. Rho then recruits Src tyrosine kinase and cytsokeletal proteins such as vinculin. Src, in turn, downregulates Rho activity. IpaA can bind vinculin and is also involved in the depolymerization of actin at the later stages of *Shigella* entry. By contrast, *Salmonella* entry does not require Rho. Cdc42 and Rac are directly activated by SopE and SopE2, which have guanine nucleotide exchange factor (GEF) activity. Furthermore, the inositol phosphatase SopB/SigD also activates Cdc42, but indirectly. SipA and SipC are cytoskeleton-altering effectors, the actions of which are cooperative. SipA binds directly to F-actin to modulate the actin-bundling (crosslinking) activity of T-plastin. SipC is involved in the nucleation of actin polymerization. The membrane ruffling response is downregulated by the GTPase-activating protein (GAP) SptP, which inactivates Cdc42 and Rac.

GTPASE-ACTIVATING PROTEIN (GAP). Protein that inactivates small GTP-binding proteins by increasing their rate of GTP hydrolysis.

Uninvited guests: bacterial invasion. Induction of bacterial invasion is also a multifactorial process involving the translocation of several effectors. *Salmonella*- and *Shigella*-induced invasion are characterized by massive cytoskeletal rearrangements at the plasma membrane and membrane ruffling. Often, more than one bacterium is engulfed in a process akin to MACROPINOCYTOSIS. Considering the resemblance of this process to the membrane ruffling that is induced by growth factors, it is perhaps not surprising that the *Salmonella* and *Shigella* effectors required for bacterial entry target the same subset of signalling molecules: small GTPases of the Rho family and Src family tyrosine kinases (FIG. 2).

Bacillary dysentery is caused by the highly contagious pathogen *Shigella*. This bacterium invades and persists within the colonic epithelium[55]. Its invasive nature is due to a large (200 kb) virulence plasmid[56] that encodes a type III secretion system and its effectors. At least five effectors secreted by the mxi-spa type III secretion system, IpaA, IpaB, IpaC, IpaD and IpgD, have been directly implicated in the events associated with *Shigella* invasion. As a complex, IpaB and IpaC can bind the fibronectin receptor, $\alpha_5\beta_1$ integrin[57], but the purpose of this interaction is not known. Additionally, IpaB alone can bind to CD44 on epithelial cells, a surface receptor that normally binds hyaluronic acid[58]. Latex beads coated with an IpaB–IpaC complex are sufficient to promote membrane ruffling, actin polymerization and subsequent internalization into epithelial cells[59]. Furthermore, the IpaB–IpaC complex is involved in pore formation in the host cell membrane, through which bacterial effectors are translocated[60]. The carboxy-terminal domain of IpaC is indirectly involved in actin polymerization[61], whereas the amino terminus participates in the formation of lamellipodia in a Rac- and Cdc42-dependent process[62]. The mechanism of Rac and Cdc42 activation by IpaC remains unknown because IpaC does not have GEF activity on these GTPases[63]. Likewise, although activation of Rho proteins is also essential for the *Shigella* entry process[64], the bacterial trigger remains unknown. During focal contact formation, Rho recruits ezrin and the tyrosine kinase Src to the site of bacterial entry. As part of a regulatory loop, the Src activity then downregulates Rho function. It has been proposed that a similar pattern occurs during *Shigella* entry[63]. IpaA directly binds the amino terminus of the cytoskeletal protein vinculin and induces its association with F-actin to depolymerize actin filaments at the base of the phagocytic structure engulfing the invading *Shigella*[65]. Other mammalian cytoskeletal proteins recruited during *Shigella* entry are cortactin, FAK and paxillin. Recently, IpgD was found to associate with IpaA after bacterial secretion, and contribute to the formation of entry focus structures[66]. So *Shigella* entry into nonphagocytic cells is a complex process that involves the coordinated action of numerous bacterial effectors.

Salmonella induces its uptake by host cells with the help of a similar type III secretion system. Additionally, many of the *Salmonella* and *Shigella* bacterial effectors involved in invasion are homologous, but do not necessarily share similar functions. In the case of *Salmonella*, SipA, SipC, SopE and SopE2, SopB/SigD and SptP are collectively involved in bacterial uptake. Microinjection of SopE into cultured epithelial cells causes membrane ruffles in conjunction with cytoskeletal rearrangements[67]. SopE and its homologue SopE2 both have GEF activity and can directly activate Cdc42 and Rac1 to initiate such a ruffling response[67]. Recent work using polarized epithelial cells has shown that the contribution of these GTPases to *Salmonella* entry is dictated by the port of entry: both Cdc42 and Rac1 are activated during basolateral internalization, but only Rac1 is activated in a SopE-dependent manner during apical internalization[68]. Despite the lack of sequence similarity between SopE/SopE2 and eukaryotic GEFs, biochemical analysis of SopE suggests that it is a close mimic of its mammalian counterpart[69]. The inositol phosphate polyphosphatase activity of SopB/SigD was recently shown to contribute to actin cytoskeleton rearrangements through activation of Cdc42, but not Rho[70]. This effect is indirect and probably driven by local changes in phosphoinositide levels. Rather than modifying the activity of signalling molecules, both SipA and SipC interact directly with the cytoskeleton to mediate cytoskeletal changes. SipA binds directly to F-actin[71] to modulate the actin-bundling activity of fimbrin[72] and enhance the rearrangements of the actin cytoskeleton that are associated with bacterial invasion. SipC is inserted into the host cell membrane[73] and is essential for *Salmonella* internalization, but seems to act differently from its *Shigella* homologue, IpaC. Recently, the amino terminus of SipC was shown to have actin-bundling (crosslinking) activity, whereas the carboxyl terminus is involved in nucleation of actin polymerization[74]. The actions of SipA and SipC are cooperative[75], and neither SipA nor SipC has sequence homology to known eukaryotic actin-binding proteins.

As with all complex cellular events, regulation of the *Salmonella* invasion is finely tuned. SptP is a modular protein with two domains that have distinct functions. The carboxyl terminus of SptP is homologous to eukaryotic tyrosine phosphatases and YopH from *Yersinia* (see above). Although this domain can mediate disruption of the host cell cytoskeleton independently of the amino terminus, its function is still unclear[76]. The amino-terminal domain, on the other hand, acts as a GAP for Rho proteins[77]. SptP stimulates the release of GTP from Cdc42 and Rac1, thus switching off their activity, and consequently reverses the actin cytoskeleton changes associated with membrane ruffling. So SptP antagonizes the actions of SopE and SopE2. It is not yet known how these two effectors are temporally regulated. In an example of convergent evolution, despite a much smaller overall size and different GAP domain fold structure, SptP and eukaryotic GAPs interact with similar residues of Rac1 through a conserved arginine finger loop[78].

Bacterial invasion by *Salmonella* and *Shigella* are excellent examples of the sophisticated ploys that pathogens use to attain their goals. In this case, the

MACROPINOCYTOSIS
Actin-dependent process by which cells engulf large volumes of fluids.

GUANINE NUCLEOTIDE EXCHANGE FACTOR
(GEF). A protein that facilitates the exchange of GDP (guanine diphosphate) for GTP (guanine triphosphate) in the nucleotide-binding pocket of a GTP-binding protein.

coordinated action of many bacterial proteins coerces the host cell to induce membrane ruffling and allow bacterial entry. These bacteria induce phenotypically similar responses at the host cell membrane, with overlapping signalling pathways and cytoskeletal components being involved. However, the two processes are also mechanistically distinct. The three Rho GTPases, Cdc42, Rac and Rho, are all required for *Shigella* entry, whereas inhibition of Rho has little effect on *Salmonella* entry, which seems to preferentially use Cdc42- or Rac1-dependent pathways, depending on the cell type. This exemplifies how bacteria can take advantage of the complexity and redundancy in host signalling pathways involved in cytoskeletal rearrangements.

Masters of disguise: hiding in a vacuole

When entering host cells through phagocytosis, intracellular pathogens place themselves at the mercy of the host's degradative machinery. As a retaliatory measure, these same pathogens have developed several strategies to survive within host cells. Pathogens such as *Listeria*, *Shigella*, *Rickettsia* and the protozoan parasite *Trypanosoma cruzi*, avoid the harsh environment of their intracellular vacuole by rapidly degrading the vacuolar membrane. This allows their escape to the cytosol where they can freely replicate[79] and use the host cytoskeletal machinery for intracellular movement[80]. *Legionella pneumophila*, *Brucella* spp., *Chlamydia* spp. and *Toxoplasma gondii*, on the other hand, escape the degradation by generating a safe replicative niche located in the biosynthetic pathway[81]. Whereas *L. pneumophila* and *Brucella* spp. use type IV secretion machineries to translocate the required effectors[82,83], *Chlamydia* seems to tailor its replicative niche by inserting numerous proteins (delivered through a type III secretion system) into the inclusion membrane[84].

By contrast, other intracellular pathogens replicate within the phagocytic vacuole. Phagosome maturation occurs by several transient fusion events with early endosomes, late endosomes and lysosomes, leading to a mature phagolysosome that is fully competent for killing invading pathogens[85]. Amazingly, some pathogens such as *Leishmania* spp. and *Coxiella burnetii* have evolved to withstand the harsh acidic environment of the phagolysosome[85,86]. As an alternative tactic, some pathogens avoid destruction by taking advantage of the multistep nature of the endocytic

Table 2 | Diverse intracellular transport of microbial pathogens

Pathogen	Origin of the vacuole	Host proteins in the vacuole	Block in acidification	Interactions	References
Mycobacterium spp.	Early endosome	Rab5 CD63 (low) LAMP (low) Cathepsin D TfR No Rab7 No v-ATPase	Yes	Endosomal recycling pathway	87,115,116
Salmonella typhimurium	Endosome	LAMP CD63 Rab7 V-ATPase No Cl-M6PR	No	Endocytic pathway	81, 89, 117
Legionella pneumophila	ER	No cathepsin D Dot-dependent: no LAMP Dot-independent: LAMP	Yes	Biosynthetic pathway Mitochondria (transient) Autophagy pathway ER (stable)	87, 118–120
Brucella abortus	ER	Sec61β Calnexin Calreticulin	No	Autophagy (transient) ER (stable) Secretory pathway?	121
Chlamydia spp.	?	None	Yes	Mitochondria (*C. psittaci*) Glycosphingolipid synthesis pathway	87, 122, 123
Coxiella burnetii	Phago-lysosome	LAMP Cathepsin D	No	Endocytic pathway	86, 122
Toxoplasma gondii	?	None	Yes	Mitochondria ER (transient)	87, 124
Leishmania spp. promastigote	Early endosome	LAMP No Rab7	No	Endocytic pathway	125
Leishmania spp. amastigote	Lysosome	LAMP Rab7 V-ATPase Cl-M6PR Cathepsin D	No	Endocytic pathway	85

v-ATPase, vacuolar ATPase; Cl-M6PR, cation-independent mannose 6-phosphate receptor; ER, endoplasmic reticulum; LAMP, lysosomal-associated membrane protein 1 and/or 2; TfR, transferrin receptor.

pathway to arrest the maturation of their own vacuole. So far, such effects have been attributed to either the pathogen-induced maintenance or the exclusion of specific host cell regulatory components of the endocytic/phagocytic pathways. For instance, *Mycobacterium* prevents fusion of its vacuole with lysosomes to arrest the maturation of its vacuole at the level of early endosomal organelles[87](also see the Review by David G. Russell on page 569 of this issue). *Toxoplasma gondii* also seems to exclude most of the plasma membrane proteins from its vacuole, thus interrupting maturation at an early stage along the endocytic pathway[87]. The specific pathogenic proteins involved in these instances are not yet known.

Comparatively more is known about the maturation of the *Salmonella*-containing vacuole (SCV). SCVs interact with early endosomes and then acquire a subset of lysosomal markers by interacting with intermediate vesicles of the late endocytic pathway but not with lysosomes[88]. *Salmonella* is unusual in having two type III secretion systems: the SPI-1 type III secretion system, which is involved in invasion (see above); and the SPI-2 system, which is required for intracellular survival. The SPI-2 system translocates effectors across the SCV to modulate the host cell functions necessary for intracellular survival. For example, SifA, an effector translocated into host cells by the SPI-2 system, is necessary for maintaining the integrity of the SCV[89]. SifA was initially identified as being necessary and sufficient for the formation of filamentous endosomal structures, known as *Salmonella*-induced filaments (Sifs), in infected epithelial cells[90,91]. This effector probably controls Rab7-dependent membrane fusion events that allow recruitment of membrane to the SCV during bacterial replication, although its precise mode of action is unknown[89]. Another SPI-2 effector protein, SpiC, is secreted by *Salmonella* into the host cell cytosol where it inhibits the fusion of SCVs with lysosomes and endosomes and also disrupts vesicle transport[92]. As for invasion, survival of *Salmonella* within a vacuole is surely dependent on the coordinated actions of many effectors, with the identity of most yet to be discovered.

Although very few of the bacterial effectors involved in vacuole modification have been identified, it is already obvious that their actions are diverse. There is no integrated model to describe how pathogens use host cell transport pathways to create a safe replicative niche. The variance in survival strategies of intracellular pathogens is shown by the assorted subcellular marker composition of their replicative vacuoles (TABLE 2). Pathogens can escape the vacuole, live within the phagolysosome, or create their own privileged niche. Regardless of the means by which it is achieved, by breaking and altering the lines of communication within the host cell, pathogens can survive and replicate undetected within the protected niche of a host cell.

Are pathogens masters of their own fate?

An important question is whether intracellular pathogens are in control of their own intracellular fate, regardless of their mode of entry. At present, there does not seem to be an unequivocal answer. For example, the host cell type, specific pathogen and activation state of the cell can all influence the outcome of this interaction.

Legionella enters macrophages by COILING PHAGOCYTOSIS under non-opsonic conditions. However, under opsonic conditions, entry is by conventional phagocytosis[93]. Regardless of these dissimilar routes of entry, *Legionella* is transported to a replicative vacuole. Similarly, in macrophages, the intracellular transport of *Salmonella* is independent of the mechanism of bacterial entry[94]. So, at least for these two cases, modification of the intracellular vacuole occurs independently of the mechanism of bacterial entry.

By contrast, fusion incompetence of the *T. gondii* vacuole is determined by the mechanism of parasite entry. Entry after binding the Fcγ-R of IgG-coated parasites routes the parasitic vacuole to mature along the endocytic pathway[95]. As a result, *T. gondii* is transported to phagolysosomes and killed. Similarly, when *Leishmania major* PROMASTIGOTES enter macrophages after binding the CR3 receptor, they are efficiently killed. However, these protozoans survive and replicate intracellularly when they bind the CR1 receptor[96]. In another example of the route of entry determining fate, antibody-opsonized *E. coli* are killed much more efficiently than *E. coli* internalized through a FimH-mediated mechanism by bone-marrow-derived macrophages[27]. The vacuoles surrounding FimH-internalized and antibody-opsonized bacteria are different. Comparatively, FimH-internalized *E. coli* reside in a much tighter-fitting vacuole that acidifies to a much lesser degree and is attenuated for the intracellular release of free radicals[27].

Therefore, individual entry pathways can, in some instances, influence the intracellular survival capacity of the pathogen, but this is not always the case. Pathogens that share a common entry strategy ultimately reside in different intracellular compartments — for example, *Salmonella* resides in an intracellular vacuole, *Shigella* and *Listeria* are cytoplasmic, and *Yersinia* are delivered to lysosomes. Further detailed investigation of pathogen–host interactions will enlighten us as to whether the bacterium or the host is in control and under what conditions. Despite the infancy of this area of research, it is already obvious that the outcome is usually dictated by contributions from both the host and the pathogen.

Concluding remarks

Although the study of infectious diseases spans over 100 years, it is only recently that we have begun to appreciate the intricate interactions and delicate balance that occurs between pathogens and their host cells. Signalling pathways abound within cells, and all represent potential mechanisms by which a bacterial effector can cause profound effects within a cell with minimal activity. By being in contact with eukaryotes for millions of years, it is likely that pathogens have 'stolen' mammalian molecules and turned them into virulence factors. Alternatively, by being forced to evolve alongside mammalian hosts, convergent evolution has resulted in some virulence factors mimicking

COILING PHAGOCYTOSIS
Internalization of a particle through enclosure by a single finger-like projection that coils around it.

PROMASTIGOTE
Flagellated form of *Leishmania* carried by an insect vector and injected into the bloodstream of the host.

the host cell function, but showing no apparent structural similarity. Once a pathogen has developed a molecule or process that provides a successful strategy, this virulence factor is rapidly disseminated by mobile genetic elements to other pathogens where it can be incorporated into their own pathogenic repertoire and modified further. As molecular studies progress on pathogen–host interactions, the convergence of cell biology and microbiology becomes imperative to understanding pathogenic mechanisms. Hopefully this knowledge can be applied to the continuing fight against infectious diseases worldwide.

Links

DATABASE LINKS Internalin | E-cadherin | InlB | Met tyrosine kinase | C1q | HGF | PI3K | invasin | $\alpha_3\beta_1$ | $\alpha_4\beta_1$ | $\alpha_5\beta_1$ | $\alpha_6\beta_1$ | $\alpha_v\beta_1$ | fibronectin | talin | α-actinin | FAK | CAS | Rac1 | *fimH* | CD48 | Fcγ-R | CR1 | Cdc42 | RhoA | YopH | Fyb | SKAP-HOM | Crk | YopE | ExoS | ExoT | Src | IpaA | IpaB | IpaC | IpaD | IpgD | CD44 | ezrin | vinculin | cortactin | paxillin | SipA | SopB | SigD | SptP | SifA | Rab7 | SpiC

FURTHER INFORMATION Finlay lab | Salmonella.org | Pathogenic *E. coli* infection mechanism | Intracellular infection by *Salmonella* | Theriot lab movie collection

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