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The cytoskeleton in cell-autonomous immunity: structural determinants of host defence

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Competing interests statement

The authors declare no competing interests.

Glossary of terms

Pyrin An inflammasome-activating protein that is not part of the nucleotide-binding and oligomerization domain (NOD)- and leucine-rich repeat (LRR)-containing protein (NLR) family. It has a PAAD (pyrin, AIM, ASC death-domain-like) domain, a DAPIN (domain in apoptosis and interferon response) domain, a PYD (pyrin amino-terminal homology domain), a tripartite motif (TRIM; containing the RING domain, B-box and coiled coil domains), and a carboxy-terminal B30.2 domain.

Inflammasomes Large oligomeric multiprotein complexes that act as signalling platforms to catalytically activate pro-caspase 1 into its active p20 and p10 subunits, promote the maturation of interleukin-1 β (IL-1 β) and IL-18, and triggers pyroptosis.

Non-canonical inflammasome activation NOD-, LRR- and pyrin domain-containing 3 (NLRP3)- and ASC-dependent activation of caspase 1 by caspase 4- mediated detection of lipo- polysaccharide that promotes caspase 1-dependent maturation of interleukin-1 β (IL-1 β) and IL-18, and triggers caspase 4-dependent pyroptosis.

Pyroptosis A form of necrotic cell death that is activated by caspase 1, caspase 4 or caspase 5 and results in inflammation; morphological features include cell swelling and the formation of membrane pores, eventually leading to cell lysis.

Autophagy A catabolic, membrane- trafficking process that involves degradation of cellular components through the actions of lysosomes.

Autolysosome The compartment resulting from the fusion of an autophagosome with a lysosome.

Actin A globular protein (G-actin) that forms microfilaments (F-actin) by polymerizing in an ATP-dependent manner.

Microtubules Highly dynamic cylindrical filaments made from α - and β -tubulin heterodimers. They are formed by polymerization of tubulin in a GTP-dependent manner.

Intermediate filaments A heterogeneous protein family that contains both nuclear and cytoplasmic proteins, which assemble into filaments intermediate in size between smaller microfilaments and larger microtubules.

Septins Conserved family of GTP-binding proteins that assemble to form hetero-oligomeric complexes, filaments and rings.

RHO family GTPases Small (~21 kDa) GTP-hydrolysing enzymes that cycle between their active (GTP-bound) and inactive (GDP-bound) forms and act as molecular switches.

Type III secretion system (T3SS). A needle-like structure, also called the injectisome, used by Gram-negative bacteria to secrete proteins from the bacterial cell into the eukaryotic cell.

Guanine nucleotide exchange factor (GEF). Factor that activates monomeric GTPases by stimulating the release of GDP to allow binding of GTP; some GEFs can activate multiple GTPases, whereas others are specific to a single GTPase.

Druggable human genes A collection of human genes — typically from large gene families such as heterotrimeric G protein-coupled receptors, protein kinases, ion channels and ubiquitin ligases — predicted to bind small molecules that could alter their function in a therapeutically beneficial way.

Cytochalasin D A fungal metabolite that binds to actin filaments and is used to block the polymerization and elongation of actin.

Resistance genes (R-genes). Genes in plants that encode proteins with nucleotide-binding oligomerization domain (NOD) and leucine-rich repeat (LRR) domains, and amino-terminal interaction motifs such as Toll-IL-1 receptor (TIR) motif or coiled-coil domains.

Switch I regions Flexible loops present in RHO GTPases that contain residues that coordinate Mg²⁺ and allow binding to GTP or GDP.

Necrosis A form of lytic cell death that frequently results from toxic injury, hypoxia or stress, which is in contrast to genetically controlled programmed cell death pathways such as apoptosis. This form of cell death is usually pro-inflammatory.

CARD9-, BCL-10- and MALT1-containing complex (CBM complex). A complex containing caspase activation and recruitment domain 9 (CARD9), B cell lymphoma 10 (BCL-10) and MALT lymphoma-associated translocation protein 1 (MALT1) that triggers nuclear factor- κ B signalling downstream of immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors in myeloid cells.

Frustrated phagocytosis The phenomenon of incomplete or stalled phagocytosis: that is, when a phagocyte is unable to completely engulf and internalize a target particle.

Filopodia Actin-rich, slender projections at the leading edge of motile cells with roles in sensing, migration and cell-cell interactions.

Lamellipodia Actin-rich projections at the leading edge of motile cells that coexist with filopodia and are essential for cellular movement along a substratum.

Diffusion barrier A mechanism to compartmentalize cellular membranes into separate domains; diffusion barriers can be found in continuous membranes, such as the plasma and endoplasmic reticulum membranes, and restrict the diffusion of membrane-associated proteins.

Rapamycin A pharmacological inhibitor of mammalian target of rapamycin (mTOR), which is a central regulator of cell metabolism, growth, proliferation and survival. It is used to induce autophagy or when used at high doses, it can suppress the immune system.

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Abstract

Host cells use antimicrobial proteins, pathogen-restrictive compartmentalization and cell death in their defence against intracellular pathogens. Recent work has revealed that four components of the cytoskeleton — actin, microtubules, intermediate filaments and septins, which are well known for their roles in cell division, shape and movement — have important functions in innate immunity and cellular self-defence. Investigations using cellular and animal models have shown that these cytoskeletal proteins are crucial for sensing bacteria and for mobilizing effector mechanisms to eliminate them. In this Review, we highlight the emerging roles of the cytoskeleton as a structural determinant of cell-autonomous host defence.

Cell-autonomous immunity, which is defined as the ability of a host cell to eliminate an invasive infectious agent, is a first line of defence against microbial pathogens ¹. It relies on antimicrobial proteins, specialized degradative compartments and programmed host cell death ^{1–3}. Cell-autonomous immunity is mediated by tiered innate immune signalling networks that sense microbial pathogens and stimulate downstream pathogen elimination programmes. Recent studies on host– microorganism interactions show that components of the host cell cytoskeleton are integral to the detection of bacterial pathogens as well as to the mobilization of antibacterial responses (FIG. 1).

To detect bacteria, host cells survey extracellular, vacuolar and cytosolic spaces using specialized sensor proteins that trigger transcriptional and post-translational responses. Based on sequence conservation, these sensor proteins are broadly classified as Toll-like receptors (TLRs) ⁴, retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) ⁵, nucleotide-binding and oligomerization domain (NOD)- and leucine-rich repeat-containing proteins (NLRs) ^{6,7}, absent in melanoma (AIM2)-like receptors (ALRs) ⁸, C-type lectin receptors (CLRs) ⁹ and sequestosome 1-like receptors (SLRs) ^{10,11}. The ligands and signals that they detect, and the molecular mechanisms that are involved in their activation and downstream signalling, are well characterized and reviewed elsewhere ^{4–11}. Transcriptional changes are among the most important responses downstream of TLRs, RLRs and ALRs, and they are brought about via the nuclear factor- κ B (NF- κ B), mitogen-activated protein kinase (MAPK) or interferon (IFN)-regulatory factor (IRF) pathways ^{12–14}. In turn, newly transcribed genes, including IFN-stimulated genes and those encoding pro-inflammatory cytokines, have crucial roles in cell-intrinsic control of bacterial pathogens and the activation of adaptive immunity ^{15,16}.

Pyrin and some NLRs and ALRs detect bacterial infection and assemble signalling scaffolds called inflamma some, which leads to the activation of caspase 1 (REFS ^{7,17}). Caspase 1 proteolytically processes the pro-inflammatory cytokines interleukin- 1β (IL- 1β) and IL-18, and controls their secretion from cells ^{7,17}. In some contexts, such as during infection by Gram-negative bacteria that invade the cytosol, related inflammatory caspases — namely, caspase 4 (previously known as caspase 11 in mice) and caspase 5 — are directly activated

by binding to bacterial lipopolysaccharide (LPS) 18,19 . In response to cytoplasmic LPS, caspase 4 stimulates the NLR sensor molecule NOD-, LRR- and pyrin domain-containing 3 (NLRP3), which induces caspase 1 activation and cytokine maturation 20–23 . This caspase 4-dependent non-canonical inflammasome activation is distinct from most other scenarios in which bacterial sensing by NLRs, ALRs or pyrin results in canonical caspase 1 activation independently of caspase 4 (REFS 20–23) . In addition, caspase 1, caspase 4 and caspase 5 can trigger pyroptosis, a lytic form of host cell death that has emerged as a cell-autonomous mechanism of preventing the establishment and spread of infection by eliminating infected cells 18,19,22–24.

The SLRs, such as p62 (also known as sequestosome 1) and nuclear dot protein 52 (NDP52; also known as CALCOCO2), share a conserved ubiquitin-binding domain 10,11 . SLRs initiate autophagy by detecting ubiquitylated substrates and/or damaged membrane remnants that are associated with bacteria that rupture vacuoles and escape into the cytosol 10,11 . In this way, SLRs promote host defence by selective targeting of cytosolic bacteria to an autolysosome²⁵.

In this Review, we discuss how cytoskeletal components govern cellular self-defence through activation and execution of innate immune signalling by the families of sensors and receptors described above. We also discuss how cellular compartmentalization by the cytoskeleton enables defence strategies to eliminate bacterial pathogens. Actin²⁶ , microtubules²⁷ , intermediate filaments²⁸ and septins²⁹ are four major cytoskeletal components of vertebrate cells, and their dynamic reorganization underlies a wide range of cellular processes that require strict compartmentalization (BOX 1) . Recent work has shown that cytoskeletal rearrangements during bacterial infection promote cell-intrinsic immunity by initiating bacterial sensing, enabling subcellular niches for differential innate immune signalling, providing scaffolds for compartmentalization of pathogens, and executing antibacterial programmes such as autophagy and host cell death (FIG. 1) . Here, we discuss these emerging roles of the cytoskeleton in innate immunity and cell-autonomous restriction of bacterial pathogens.

Sensing bacterial pathogens

Bacterial pathogens have evolved various mechanisms to manipulate host cytoskeletal proteins to promote their intracellular replication and survival 30 (BOX 2) . Recent studies have shown that, to promote immunity, innate immune sensors recognize modifications to the host cytoskeleton that are induced by bacteria (TABLE 1) . In addition, cytoskeleton-dependent compartmentalization of signalling events is required to sense invading bacteria. In this section, we discuss advances in our understanding of these areas, as well as the concept of extracellular F-actin as a ‘danger signal’.

Sensing of cytoskeleton dynamics by NLRs

During invasion of host cells, bacteria induce changes to RHO family GTPases that are detected by the NLR protein NOD1 (REF. 31) (FIG. 2a; TABLE 1) . *Salmonella enterica* subsp. *enterica* serovar Typhimurium uses a type III secretion system (T3SS) to secrete the *Salmonella* pathogenicity island 1 (SPI-1) effector SopE into host cells 32 . SopE, which is a

bacterial guanine nucleotide exchange factor (GEF) for the GTPases RAC1 and cell division cycle 42 (CDC42), induces actin-dependent membrane ruffling during invasion 32 . NOD1 forms multiprotein complexes with heat shock protein 90 (HSP90), SopE and activated RAC1 or CDC42 (REF. 31) . Thus, NOD1 detects SopE-induced changes to RAC1 and CDC42, and acts as a guardian of the cytoskeleton during *S. Typhimurium* infection (FIG. 2a; TABLE 1) . NOD1 is also a cytosolic receptor for peptidoglycan fragments containing the dipeptide d-glutamyl-meso-diaminopimelic acid (iE-DAP), which is primarily found in the cell wall of Gram-negative bacteria but also in some Gram-positive bacteria such as *Listeria* spp. and *Bacillus* spp. 6 . NOD1-binding to iE-DAP-containing peptides induces NF- κ B- and MAPK-dependent gene transcription and inflammatory responses through the activation of receptor-interacting protein 2 (RIP2; also known as RIPK2) 6 (FIG. 2a) . When cells are exposed to iE-DAP ligands in the absence of bacterial GEFs, RAC1 activation by the host cell is still required for NOD1-dependent NF- κ B signalling, but the mechanism for this is unknown 31 (FIG. 2a) . In addition, exogenously expressed constitutively active forms of RHOA, RAC1 or CDC42 — which are unable to hydrolyse GTP — form complexes with NOD1 and induce RIP2-dependent NF- κ B activation 31 . This indicates that NOD1 responds to active forms of these three RHO GTPases. Thus, NOD1 mediates host defence in two distinct ways: by detecting peptidoglycan fragments and by sensing bacterial modification of RHO proteins.

The mechanistic links between NOD1 and the actin cytoskeleton are also clear from studies on *Shigella flexneri* invasion of host epithelial cells (FIG. 2b) . The localization of NOD1 at the plasma membrane and its activation by *S. flexneri* are dependent on actin 33 . The RHOA GEF GEF-H1 is required for cell invasion by *S. flexneri*, and GEF-H1 interaction with NOD1 is required for the detection of iE-DAP ligands and of secreted *S. flexneri* effectors such as IpgB2 (a RHOA GEF) and OspB (a protein kinase) 34 (FIG. 2b) . NOD1–GEF-H1-induced activation of RHOA triggers NF- κ B activation and IL-8 secretion via the actin regulatory RHOA target RHO-associated protein kinase 1 (ROCK1) 34 (FIG. 2b) . Interestingly, unlike NOD1 sensing of iE-DAP ligands, NOD1-dependent NF- κ B activation by IpgB2 and OspB is independent of RIP2 but requires ROCK1 (REF. 34) (FIG. 2b) . Hence, the downstream signalling mechanisms underlying NOD1-mediated sensing of *S. flexneri* effector proteins are different from those underlying NOD1-mediated sensing of peptidoglycan ligands. A short interfering RNA (siRNA) screen against druggable human genes identified Slingshot homologue 1 (SSH1; a cofilin phosphatase) as essential for iE-DAP ligand-induced NOD1-dependent secretion of IL-6 and IL-8 (REF. 35) (FIG. 2b) . Cofilin, which promotes actin depolymerization, is inhibited by LIM domain kinase 1 (LIMK1)-dependent phosphorylation 36 ; SSH1 counteracts this effect via cofilin dephosphorylation (FIG. 2b) . As a result, SSH1 promotes F-actin disassembly and G-actin recycling in response to iE-DAP ligands or invasion by *S. flexneri* 35. Treatment of cells with cytochalasin D, which is an inhibitor of actin polymerization, mimics SSH1 silencing and impairs NOD1-dependent NF- κ B activation 35 . Taken together, NOD1 signal transduction is intimately linked to actin dynamics and manipulation of these dynamics by invasive bacteria. Studies using mice have revealed important roles for the detection of cell wall-associated iE-DAP by NOD1 in protection against *Helicobacter pylori* and *Legionella pneumophila* infection 6 . In the case of *S. Typhimurium*, NOD1 is essential for the

detection of SopE and inflammation in the caecum of infected mice 31 . In addition, NOD1 signalling in dendritic cells (DCs) of the intestinal lamina propria also defends against SPI-1-deficient *S. Typhimurium* (which does not express SopE) 37 . Enhanced systemic infection in Nod1 $-/-$ mice infected with SPI-1-deficient *S. Typhimurium* points to a DC-specific role for NOD1 in defence of the lamina propria against this pathogen 37 .

Bacterial peptidoglycan fragments containing muramyl dipeptide (MDP) are detected by NOD2, which is an NLR that is closely related to NOD1 and also activates NF- κ B and MAPK via RIP2 (REF. 6) . Studies using Nod2 $-/-$ mice have highlighted the role of NOD2 in immunity to *Listeria monocytogenes*, *Yersinia pseudotuberculosis* and adherent-invasive *Escherichia coli* 6 . In monocytes treated with MDP, NOD2 is recruited to membrane ruffles containing F-actin via RAC1 and RHO GEF 7 (ARHGEF7; also known as β -PIX) 38 . Membrane recruitment of NOD2 promotes its inter-action with ERBB2-interacting protein ERBIN (also known as LAP2), which is a negative regulator of NOD2 38 . Thus, the inhibition of RAC1 enhances MDP-induced NF- κ B activation and IL-8 secretion. In agreement with this, treatment of cells with cytochalasin D enhances NOD2 signalling 39 . As in the case of NOD1 signalling 34 , GEF-H1 promotes NOD2-induced NF- κ B activation and the secretion of tumour necrosis factor (TNF) and IL-6 (REF. 40) . However, unlike NOD1 signalling 34 , GEF-H1 activity is actin independent and promotes NOD2 responses by mediating SRC tyrosine kinase-dependent phosphorylation of RIP2 (REF. 40) . NOD2-dependent activation of NF- κ B pathways also relies on NOD2 interactions with the intermediate filament vimentin 41 . Inhibition of vimentin using withaferin A leads to a redistribution of NOD2 from the plasma membrane to the cytosol in colonic epithelial cells and abrogates NOD2-dependent signalling 41 . In this case, NOD2–vimentin interactions are crucial for autophagy and for the control of a Crohn disease-associated strain of adherent-invasive *E. coli* 41 . These studies indicate important roles for the cytoskeleton in regulation of NOD2 signalling by controlling its localization 38–41 .

Taken together, modulation of cytoskeleton dynamics can trigger immune responses via NOD1 and NOD2. These studies are among the first to suggest the role of mammalian NLRs in guarding against changes to host proteins in a manner similar to the plant immunity-related resistance genes (R-genes) 42 . To mount immune responses in plants, members of expanded families of polymorphic R-genes detect infection-induced alterations of cellular proteins. The plant R-genes and mammalian NLRs have the NOD and leucine-rich repeat (LRR) regions in common, which points to analogous microorganism-sensing mechanisms in diverse eukaryotes, and they represent a striking example of innate immune proteins that have undergone convergent evolution 42 .

Sensing of cytoskeleton dynamics by pyrin

Pyrin (encoded by Mediterranean fever gene (MEFV) 43) is the first non-NLR guardian of host proteins, which, similarly to some NLRs and ALRs, has an amino-terminal pyrin homology domain (PYD) that enables homotypic inter actions. Mutations in MEFV are associated with increased activation of caspase 1 and IL-1 β , which is responsible for familial Mediterranean fever syndrome 43 . Recent work has shown that bacteria-induced covalent changes to RHO GTPases (RHOA, RHOB and RHOC) are detected by pyrin to assemble

inflammasomes 44 (FIG. 2c; TABLE 1). Inflammasome assembly requires pyrin interactions with the adaptor protein ASC, which has a PYD and caspase activation and recruitment domain (CARD) for interactions with NLRs, ALRs and caspase 1 (REF. 7). The CARD in ASC interacts with the CARD in caspase 1, resulting in oligomeric assembly of inflammasome complexes 7 (FIG. 2c). Pyrin is a versatile molecular sensor of toxin-induced covalent modifications to amino acids within the switch I regions of RHO GTPases 44. ADP-ribosylation, glucosylation, adenylation (also called AMPylation) and deamidation of RHO GTPases all activate the pyrin inflammasome 44 (TABLE 1). Mechanistically, pyrin–RHO interactions are not required for pyrin inflammasome assembly 44. These observations support an indirect mode of sensing bacterial disruption of RHO GTPases and actin signalling. Various bacteria — such as *Clostridium difficile*, *Clostridium botulinum*, *Vibrio parahaemolyticus* and *Histophilus somni* — can be detected by pyrin as a consequence of bacterial toxin-induced modifications of RHO GTPases 44 (TABLE 1). *Burkholderia cenocepacia* activates pyrin inflammasomes via an unknown secreted protein that deamidates RHOA, and macrophages deficient in pyrin (Mefv $-/-$) are unable to restrict intracellular growth of *B. cenocepacia* in vitro 44,45. The role of pyrin in host defence is also clear from experiments with Mefv $-/-$ mice, which exhibit reduced lung inflammation when infected with *B. cenocepacia* 44. *Francisella tularensis*, a cytosol-resident pathogen, uses an actin-dependent ‘looping phagocytosis’ mechanism to enter phagocytes 46. The detection of *F. tularensis* by pyrin activates caspase 1 (REF. 47); however, the role of RHO GTPase modifications has not been determined. Pyrin can also bind actin with low affinity and be recruited to sites of actin polymerization (for example, membrane ruffles and *L. monocytogenes* actin tails) 48. Intriguingly, mouse pyrin, which lacks the carboxy-terminal B30.2 domain present in human pyrin 49, is dispensable for the detection of *F. tularensis* and *L. monocytogenes* 49–51; in mice, AIM2 has an important role in activating caspase 1 after binding to DNA from cytosolic *F. tularensis* 50,51 and *L. monocytogenes* 52. Together, these examples show that human and mouse pyrin have emerged as sensors of RHO GTPase modifications by extracellular and intra-cellular bacterial pathogens. Pyrin activates both cytokine release and cell death, and it is therefore an important mediator of cell-extrinsic and cell-intrinsic immunity.

More recently, a broad role for pyrin in guarding the actin cytoskeleton has emerged from studies in Wdr1 rd/rd mice, which express hypomorphic alleles of the gene encoding the actin-depolymerizing cofactor WD repeat-containing protein 1 (WDR1; also known as AIP1) 53,54. Wdr1 rd/rd mice display spontaneous activation of caspase 1 in monocytes and systemic IL-18-dependent inflammation 53. Strikingly, these phenotypes are abolished when Wdr1 rd/rd mice are crossed with either Casp1 $-/-$, Il18 $-/-$ or Mefv $-/-$ mice 53. This indicates that disruption of actin, due to reduced WDR1 activity, induces the pyrin inflammasome and initiates IL-18-dependent autoinflammation. Therefore, pyrin is a key homeostatic sensor of actin dynamics in infectious and non-infectious contexts. In summary, NOD1, NOD2 and pyrin can monitor the host cell cytoskeleton and mount specific innate immune responses to pathogen-induced cytoskeletal rearrangements. Strikingly, the cytoskeleton also enables bacterial detection by other innate immune sensors that respond to a wide range of microbial signals: for example, NLRs, TLRs and caspase 4 (REF. 17).

Cytoskeletal roles in bacterial sensing by inflamma-somes

NLRP3, one of the best-studied inflammasome-associated NLRs, is activated by the depletion of cytosolic potassium ^{7,55}. For example, ATP-induced P2X7 receptor signalling and bacterial pore-forming toxins (for example, listeriolysin O produced by *L. monocytogenes*) activate caspase 1 via NLRP3 (REFS 7,17) (FIG. 2d). In addition, ‘sterile’ particulates that are phagocytosed by macrophages — such as alum adjuvants, monosodium uric acid (MSU) crystals and asbestos — activate NLRP3 upon rupture of vacuoles containing internalized particles ^{7,56}. Supporting a role for actin in NLRP3 activation, inhibition of phagocytosis using cytochalasin D abolished NLRP3 activation by MSU crystals ⁵⁷. Therefore, phagocytosis of sterile particulate agonists is essential for NLRP3 activation. A chemical screen revealed that inhibitors of tubulin polymerization (colchicine, nocodazole and podophyllotoxin) specifically abolished NLRP3-dependent caspase 1 activity, whereas AIM2-dependent and NOD-, LRR- and CARD-containing 4 (NLRC4)-dependent activation of caspase 1 were unaffected ⁵⁷. Colchicine was initially reported to inhibit NLRP3 activation in response to particulate MSU or calcium pyrophosphate dehydrate and not in response to the soluble agonist ATP ⁵⁸. More recently, however, microtubules were found to be involved in NLRP3 activation by both MSU crystals and ATP ⁵⁷. The reasons for this discrepancy are presently unclear. Caspase 1 activation by NLRP3 requires ASC, which can also be present on mitochondria ⁵⁷. Trafficking of mitochondria to endoplasmic reticulum (ER) sites containing NLRP3 is mediated by tubulin, and therefore tubulin-inhibiting agents abolish NLRP3 activation ⁵⁷. Furthermore, acetylation of tubulin by MEC17 (also known as ATAT1) favours mitochondrial trafficking to the ER; the deacetylase SIRT2 reduces acetylated tubulin levels and thus also controls mitochondrial trafficking ⁵⁷. Thus, a balance of tubulin acetylation–deacetylation affects mitochondrial transport, NLRP3–ASC association and downstream signalling to caspase 1 (REF. 57).

In a recent study, vimentin-deficient mice were shown to be protected against lethal challenge with LPS, bleomycin-induced acute lung injury and asbestos-induced inflammation, all of which depend on the NLRP3–caspase 1 signalling axis ⁵⁹. In macrophages, vimentin directly interacts with NLRP3 and modulates its activation in response to MSU crystals, ATP and asbestos ⁵⁹ (FIG. 2d). This study revealed an important role for vimentin in systemic inflammatory responses through effects mediated by NLRP3, caspase 1 and IL-1 β ⁵⁹. Antimicrobial and pro-inflammatory actions of NLRP3 are regulated by complex signalling networks, including reactive oxygen species (ROS) ⁵⁶, ubiquitylation ⁵⁶, guanylate-binding protein 5 (GBP5) ⁶⁰, nitric oxide ⁶¹ and mitochondrial antiviral signalling protein (MAVS) ^{62,63}. The precise role of the cytoskeleton in these signalling networks remains to be determined.

Colchicine treatment in gout — an inflammatory condition associated with NLRP3 activation by crystalline MSU — suppresses inflammation by inhibiting the NLRP3–caspase 1–IL-1 β pathway, which suggests an *in vivo* role for microtubules in NLRP3-mediated inflammation ⁶⁴. In addition, monogenic hereditary autoinflammatory conditions, collectively called cryopyrin-associated periodic syndromes, result from mutations that cause constitutive activation of NLRP3 (REF. 65). Multifactorial disorders, such as metabolic syndrome and atherosclerosis, are also associated with dysregulation of NLRP3

(REF. 66) . Therefore, future studies focusing on the precise interplay between the cytoskeleton and NLRP3 signalling should have a considerable impact on therapeutic strategies for infectious as well as non-infectious human diseases.

The cytoskeleton as a danger signal

In addition to cell-intrinsic roles of the cytoskeleton in innate immune sensing, externalized F-actin on dead cells has been identified as a danger signal 67,68 (FIG. 3a) . F-actin on the surface of cells undergoing necrosis acts as a ligand for the homodimeric C-type lectin domain family 9 member A (CLEC9A; also known as DNGR1) 69 (FIG. 3a). CLEC9A is highly expressed by DC populations involved in cross-presentation, such as mouse CD8 α + DCs and the human BDCA3 + DC subset. Actin from yeast, insects, fish, birds, mice and humans activates CLEC9A, which strongly suggests its role as an evolutionarily conserved danger signal 68 . Recent structural analyses revealed that the C-type lectin domain in CLEC9A binds three actin subunits across two protofilaments of F-actin 70. This unique interaction allows for the recognition of cell death and results in high-avidity binding, receptor crosslinking and endocytosis 70. Downstream signalling by CLEC9A uses spleen tyrosine kinase (SYK) to direct CLEC9A to non-lysosomal compartments and preserve dead cell-associated antigens 69,71 (FIG. 3a) . F-actin– CLEC9A interactions are not required for the phagocytic uptake of dead cells per se; however, CLEC9A has an indispensable role in cross-presentation of necrotic cell-associated antigens by CD8 α + DCs 69–71. For example, during vaccinia virus infection, CLEC9A is crucial for cross-presentation of viral antigens following virus-induced cell death 74. Therefore, cell death during infection promotes pathogen-associated antigen cross-presentation in a manner strictly dependent on the role of F-actin as a danger signal. An innate immune role for extracellular actin has also been found in studies using *Anopheles gambiae*, which have shown that actin strongly inhibits the replication of bacteria (for example, *E. coli* and *Staphylococcus aureus*) and parasites (such as *Plasmodium falciparum*) 75 . Bacterial toxins can also covalently modify actin: for example, by ADP-ribosylation (which can be mediated by *C. botulinum* C2 toxin and *Photobacterium luminescens* toxin complex) or by actin crosslinking (which is catalysed by, for example, *Vibrio cholerae* RTX toxin) 76 . However, it is currently unknown whether host sensors and/or receptors can directly detect such modifications.

Role of actin in CLR and TLR signalling and phago cytosis

The plasma membrane-associated actin network is indispensable for endocytosis of receptors and phagocytosis of bacteria, and both processes can determine the outcomes of innate immune signalling by CLRs and TLRs. The CLR dectin 1 (also known as CLEC7A) induces the phago cytosis of β -glucan ligand-carrying cargo and downstream activation of SYK via its immunoreceptor tyrosine-based activation motif (ITAM). SYK triggers the CARD9-, BCL-10- and MALT1-containing complex (CBM complex) to stimulate NF- κ B-dependent gene transcription 77 (FIG. 3b) . Preventing dectin 1-dependent phagocytosis in DCs using cytochalasin D results in frustrated phago cytosis and enhances the production of the pro-inflammatory cytokines TNF, IL-6 and IL-12p40 (REF. 78) (FIG. 3b) . Although TLRs are not phagocytic receptors per se, they promote the uptake of soluble antigens by endocytosis 79,80. Endocytosis of TLRs is a key determinant of downstream transcriptional responses, phagosome maturation and targeting by autophagy 79,80. TLR4 uses the myeloid

differentiation primary response protein 88 (MYD88) adaptor at the plasma membrane and the TIR domain-containing adaptor protein inducing IFN β (TRIF) adaptor when signalling after endocytosis 81 . MYD88 controls the activation of NF- κ B and MAPK, and the induction of pro-inflammatory cytokines such as TNF, whereas TRIF controls IRF-dependent transcription of type I IFNs 4 (FIG. 3c) . In plasmacytoid DCs (pDCs), actin-dependent trafficking of TLR9, an endosomal TLR that detects unmethylated CpG DNA motifs, to lysosome-like organelles is essential for IRF7-dependent induction of type I IFNs 82 (FIG. 3c) . Here, dedicator of cytokinesis 2 (DOCK2), a RAC1 GEF, is required for F-actin accumulation around TLR9 endosomes to promote their trafficking 83 . Similarly, during *S. aureus* infection, phagocytic uptake of bacteria by pDCs is essential for the induction of type I IFNs via TLR9 (REF. 84) . DC activation via TLR signalling can also proceed upon phagocytosis of apoptotic cells that contain TLR ligands or that are infected with bacteria; for example, during infection of mice with *Citrobacter rodentium*, a model used to mimic human enteropathogenic and enterohaemorrhagic *E. coli* (EPEC and EHEC, respectively) infections 85 . Therefore, whereas soluble TLR agonists trigger plasma membrane-localized receptors, endocytosis-dependent and phagocytosis-dependent signalling also shape immune responses and are likely to have important implications in the design of effective vaccines and adjuvants 80 .

TLR4-induced phagocytosis of Gram-negative bacteria, such as *E. coli*, requires the cytosolic RNA-sensor RIG-I (encoded by Ddx58 in mice) 86 . RIG-I is dispensable for phagocytosis via Fc γ or C3 receptors, and it controls TLR4-induced activation of RAC1 and CDC42 and the recruitment of the seven subunit actin-related protein 2/3 (ARP2/3) complex (which is the major actin nucleator in host cells) to bacterial phagocytic cups 86 . Thus, in response to LPS, Ddx58 $-/-$ macrophages have reduced numbers of filopodia and lamellipodia owing to inhibited F-actin dynamics 86. These defects render Ddx58 $-/-$ mice susceptible to infection by *E. coli*, pointing to a key role for RIG-I in immunity through effects on actin dynamics.

Taken together, these studies highlight the importance of the cytoskeleton in compartmentalized signalling during endocytic and phagocytic processes. Actin polymerization is clearly crucial for uptake processes; however, roles for other cytoskeletal components are starting to emerge. Interestingly, septin rings can link actin with the plasma membrane at the site of bacterial entry 87,88 and phagocytosis 89 . Here, the cytoskeleton may act as a diffusion barrier⁷² , to confine calcium responses during *S. flexneri* invasion 73 and restrict the localization of phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) during phagocytosis 90 to promote efficient internalization. Further studies will be required to clarify the precise molecular mechanisms as well as the role of membrane-associated proteins, actin-binding proteins and other cytoskeletal elements in endocytic and phagocytic processes.

Effector responses to eliminate pathogens

In addition to pathogen sensing, the cytoskeleton promotes effector mechanisms that control the intracellular fate of bacterial pathogens. In this section, we examine autophagic

elimination and lysosomal delivery of bacteria, and the role of inflammasome-related proteins in regulating cytoskeletal dynamics.

Bacterial autophagy and the cytoskeleton

Autophagy involves the initiation and elongation of double-membrane phagophores that close and sequester cargo within autophagosomes, and this process requires the coordinated action of autophagy-related (ATG) proteins that are conserved from yeast to humans 25,91 . As shown by studies using *L. monocytogenes*, *S. flexneri*, *F. tularensis*, *S. Typhimurium*, *Streptococcus pyogenes* and mycobacteria, the replication of vacuolar and cytoplasmic bacteria can be controlled by autophagy 92. Hence, bacterial autophagy (also called xenophagy) is recognized as a crucial component of cell-autonomous immunity 25,162. However, some bacterial pathogens can avoid or exploit the autophagy machinery for intracellular survival 92,93 (see Supplementary information S1 (table)). Interactions between autophagy and the cytoskeleton have been increasingly recognized 94,95, and key aspects determining the cell-autonomous control of bacterial infection are discussed here.

Recent work has shown that actin-dependent processes coincide with ubiquitylation to regulate autophagy of cytosolic bacteria 94 ; for example, autophagy of *S. flexneri* requires polymerization of actin by IcsA and host neuronal Wiskott–Aldrich syndrome protein (N-WASP), SLR-dependent detection of ubiquitylation and IcsA–ATG5 interactions 96–98 (FIG. 4a) . Interestingly, the tectonin β -propeller repeat-containing protein 1 (TECPR1), which binds to ATG5, acts as a tethering factor to promote autophagosome–lysosome fusion during selective autophagy of bacteria, and is not required for rapamycin- or starvation-induced non-selective autophagy 99,100. In the case of *L. monocytogenes*, ActA coats the bacterial surface to polymerize actin and inhibits autophagy by preventing ubiquitylation and the recruitment of p62 and NDP52 (REFS 97,101) (FIG. 4a) . These observations indicate that actin polymerization by *L. monocytogenes* and *S. flexneri* influences recognition by the autophagy machinery, yet the role of actin in bacterial autophagy can be different. Comparative analyses of cytosolic pathogens that form actin tails — including *Rickettsia* spp., *Mycobacterium marinum* and *Burkholderia* spp. — should provide a more complete understanding of the diverse roles of actin in bacterial autophagy.

Although microtubules, intermediate filaments and septins are recognized for their structural roles in autophagy 94,95 , their exact roles in controlling bacterial replication through effects on autophagy remain less clear. Microtubules promote autophagosome biogenesis, trafficking and fusion with lysosomes 95,163. Therefore, manipulation of microtubule dynamics may allow some pathogens to escape autophagy. For example, *S. Typhimurium* uses T3SS effectors acting on microtubule-associated proteins, including the SPI-2 effector SifA, to maintain integrity of the *Salmonella*-containing vacuole (SCV) 102 and thereby avoid release into the cytosol where bacteria would be targeted by autophagy. Whereas previous studies showed that autophagy restricts the replication of cytosolic *S. Typhimurium* 103–105, experiments using live-cell imaging revealed that a subset of cytosolic, SifA-deficient *S. Typhimurium* use p62 and/or the autophagy-related protein microtubule-associated protein 1 light chain 3 (LC3) for intracellular replication 106. Together, these

reports highlight SifA and microtubules as crucial determinants in the restriction or promotion of *S. Typhimurium* by autophagy.

Vimentin intermediate filaments suppress autophagy by interacting with beclin 1 (REF. 107) . To inhibit autophagy, the phosphorylation of beclin 1 by AKT promotes a beclin 1–14-3-3–vimentin complex 107. This aspect of autophagy inhibition may be exploited by *Chlamydia trachomatis*, which remodels intermediate filaments and F-actin to avoid cytosolic release 108. Disruption of vimentin or actin leads to release of bacteria into the cytosol and triggers host defence mechanisms 108 . IFN-inducible GTPases, such as GBP1 and GBP2, counteract bacterial subversion and promote autophagic delivery of *C. trachomatis* to lysosomes 109 . GBP1 is also important for cell-autonomous immunity against *L. monocytogenes* and mycobacteria, and it is involved in the delivery of antibacterial peptides to lysosomes via p62 (REF. 110) . GBP1 can bind and remodel actin filaments 111; however, a direct role for GBP1–actin interplay during infection has not yet been reported.

In the case of septins, studies have shown that cage-like structures scaffold the autophagy machinery around some actin-polymerizing bacteria, such as *S. flexneri* and *M. marinum*, to compartmentalize bacteria and prevent dissemination 96,97 (FIG. 4a) . Based on this, it has been proposed that septins control autophagosome size and shape 94, as shown for phospholipid-based liposomes in vitro 112 . Unlike examples of bacteria exploiting microtubules and intermediate filaments, whether bacteria exploit septin assembly to counteract autophagy remains to be determined. However, studies in zebrafish (*Danio rerio*) have suggested that septin cages are an important defence mechanism to clear *S. flexneri* in vivo 113.

Future investigation of autophagy–cytoskeleton interactions in vitro and in vivo promises to yield insight into fundamental processes underlying autophagy. The cytoskeleton may also be a crucial determinant in new roles that are being discovered for the autophagy machinery in host defence. For example, pathogen sensing via TLR engagement at the plasma membrane is linked to phagocytosis and delivery of bacteria to lysosomes via ATG5 and ATG7 (REF. 114) . TLR signalling has also been shown to increase bactericidal activity through mitochondrial ROS (mROS), highlighting mitochondria as a key player in the regulation of immunity 115. Recent work has shown striking parallels between the autophagy of bacteria and the autophagy of mitochondria (also called mitophagy) 116,117 , in agreement with the view that mitochondria can be considered as ancient bacteria that are present within eukaryotic cells. Taken together, it is tempting to speculate that studies of the interplay between mitochondria and the cytoskeleton will offer novel insights into cell-autonomous immunity.

Control of cytoskeleton dynamics by inflammasomes

Although the cytoskeleton has multiple roles in NLR signalling, inflammasome-related proteins are, in turn, important regulators of cytoskeleton dynamics. Studies have shown that caspase 4 (REFS 118,119) and NLRC4 (REF. 120) regulate actin assembly and dynamics in immune cells. Caspase 4 regulates actin filaments by promoting their depolymerization 118. Casp4 –/– splenocytes have defects in migration in response to CXC-chemokine ligand 12

(CXCL12; previously known as SDF1 α), CXCL13 (previously known as BLC) and KIT ligand in vitro, and Casp4 $-/-$ lymphocytes show homing defects in vivo 118 . Mechanistically, caspase 4 interacts with WDR1, which promotes actin depolymerization via cofilin 118. Thus, caspase 4 controls key migratory functions in immune cells by regulating actin dynamics (FIG. 4b) . In vitro studies have shown that caspase 4 also interacts with flightless 1, an actin-binding protein from the gelsolin family of actin-severing proteins, which allows its recruitment to the leading edge in macrophages 119 . Caspase 4 functions as a scaffold that interacts with flightless 1 (via its catalytic p30 domain 119) and WDR1 (via its CARD domain 118), which points to an important non-proteolytic role for caspase 4 in macrophages. Interestingly, flightless 1 can inhibit caspase 1 activity by acting as its substrate 119, and it is recruited to NLRP3 inflammasomes by LRR flightless-interacting protein 2 (LRRFIP2) 121 (FIG. 4b) . In addition, during *L. pneumophila* infection of macrophages, caspase 4 restricts intracellular bacterial growth by inducing actin dynamics around *L. pneumophila*-containing vacuoles and by promoting their fusion with lysosomes 122 (FIG. 4b) . This study showed that the reduced actin dynamics in Casp4 $-/-$ macrophages are a result of lower expression of flotillin 1, an actin nucleator, as well as increased phosphorylation of cofilin 122 . Together, emerging evidence highlights links between actin dynamics and host defence by inflammasome-associated caspase 4.

Recent studies have shown that bacterial LPS induces caspase 4 proteolytic activity and pyroptosis 18,22,23 . Strikingly, caspase 4 determines lethality in mouse models of LPS-induced septic shock 19,20,22 . Infection with Gram-negative bacteria that invade the cytosol, such as *Burkholderia thailandensis* and *Burkholderia pseudomallei*, results in pyroptosis as a consequence of LPS detection by caspase 4 (REF. 19) . The role of pyroptosis in immunity and inflammation is clear from studies in Casp4 $-/-$ mice, which are susceptible to *Burkholderia* spp. infection and resistant to septic shock 19,20 . Whether diminished actin dynamics in Casp4 $-/-$ mice influence susceptibility to *Burkholderia* spp. infection and septic shock has not been reported, and future studies should aim to unify the structural and proteolytic roles of caspase 4 in systemic immune responses.

NLRC4 is a caspase 1-activating NLR protein that senses flagellin or T3SS structural proteins delivered into the host cytosol by Gram-negative bacteria, such as *S. Typhimurium* 123 . A role for NLRC4 in actin dynamics was evident from reduced actin polymerization around *S. Typhimurium* in Nlrc4 $-/-$ macrophages 120 (FIG. 4c) . In wild-type cells, cytochalasin D prevented NLRC4-dependent assembly of ASC-containing inflammasome foci, which correlated with reduced bacterial invasion 120 . Interestingly, cytochalasin D also prevented assembly of ASC foci in response to transfection of recombinant flagellin, showing that NLRC4-dependent actin regulation in macrophages is independent of *S. Typhimurium* effectors that activate RHO GTPases 120 . Nlrc4 $-/-$ mice are susceptible to *S. Typhimurium*, as inflammasome-dependent processes — including actin polymerization and mROS production — drive host immunity to infection 120 . How the regulation of F-actin by NLRC4 can promote immunity against other NLRC4-activating bacteria, such as *S. flexneri*, EPEC, *L. pneumophila* and *Pseudomonas aeruginosa*, still needs to be explored. Importantly, whereas the roles of caspase 4 and NLRC4 in pro-inflammatory cytokine production and cell death have been well defined, their interactions with the cytoskeleton highlight additional roles in restricting bacterial replication that warrant future study.

Conclusions and perspectives

In this Review, we highlight the emerging roles and structural functions of cytoskeletal components in innate immunity and cell-autonomous protection against bacterial infections. Actin dynamics — which are involved in pathogen recognition and elimination by NLRs, TLRs and autophagy — are clearly the best studied. However, recent studies have also revealed key roles for microtubules, intermediate filaments and septins. The theme that emerges is one of close interplay between cytoskeletal proteins and molecules with dedicated roles in pathogen sensing and elimination. Viewed in this context, the cytoskeleton is integral to cell-autonomous immunity. Molecular events that result from cytoskeletal rearrangements in bacteria-infected cells are key initiators of transcriptional and post-translational events that execute defence strategies. These include cell-intrinsic actions of IFN-stimulated genes 15,110,124 , cell death 24 and autophagy 25,91,94 . Awaiting investigation are the roles of the cytoskeleton in other antimicrobial mechanisms underlying cell-autonomous immunity: for example, pathways recently shown to enlist proteins such as cyclic GMP–AMP synthase (cGAS) 125 , tripartite motif-containing 21 (TRIM21) 126 , complement protein C3 (REF. 127) and sex-determining region Y-box 2 (SOX2) 128 .

Pathogen-induced changes to host cell signalling have been extensively studied; however, we are only just beginning to understand how the immune system detects and responds to these changes. In light of these new data, several important questions emerge. For example, does the role of the cytoskeleton in cell-autonomous immunity differ across cell types: for example, phagocytes versus epithelial cells? Transcriptional programmes 12–14 , inflammasome activation and cell death 129 , autophagy 130 and the expression of isoforms of cytoskeletal proteins 26,27,29,131 vary across different cell types and tissues. How are covalent modifications of cytoskeletal proteins recognized by the host cell, and how do they affect innate immune signalling? Post-translational modifications of actin 132 , microtubules 133 , intermediate filaments 131 and septins 29 have been described, but their role in immunity is mostly unknown. The shape of particles determines internalization by phagocytosis 134 and actin-based motility 135 , and could have a key role in innate immune responses by the host cell. Thus, studies on the bacterial cytoskeletal proteins 136 — including MreB (actin-like) 137 , FtsZ (microtubule-like) 138 , crescentin (intermediate filament-like) 139 , and paraseptins 140 and MinCD 141 (septin-like) — should provide novel perspectives on structural determinants at the interface of host–microorganism interactions. In addition, investigating the role of cytoskeletal assemblies during infection by non-bacterial pathogens, including viruses (for example, vaccinia virus) and parasites (such as *Toxoplasma* spp.), is likely to provide complementary insights.

In addition to its role in the innate immune system, the cytoskeleton has pivotal roles in antigen presentation, T cell signalling and adaptive immune responses, as reviewed elsewhere 142,143 . Not surprisingly, therefore, increasing evidence implicates the cytoskeleton in the pathogenesis of several diseases, including neoplasia, neurodegenerative conditions and microbial infections 26,27,29,131 . Natural polymorphisms in cytoskeletal proteins are associated with hereditary syndromes with immune consequences: for example, WASP mutations cause Wiskott–Aldrich syndrome 144 , cardiac muscle α -actin 1 (ACTC1) mutations are associated with chronic inflammatory cardio myopathy 145 , gelsolin (GSN)

mutations cause amyloidosis-like diseases 146 , DOCK2 mutations cause early-onset invasive infections 164 , and septin 9 (SEPT9) mutations are linked to hereditary neuralgic amyotrophy 147 . What are the precise roles of cytoskeletal components in cell-autonomous immunity? Actin, microtubules, intermediate filaments and septins have been shown to interact in many cellular contexts 26,27,29,131 , and further study is required to determine how they work together during microbial infection. As discussed in this Review, studies using bacterial pathogens can reveal the breadth of cell-autonomous immune responses available to the host and define how cytoskeletal components contribute to each. This information should provide vital clues that improve our understanding of bacterial pathogenesis and illuminate new therapeutic strategies for human diseases in which cytoskeletal components have been implicated, such as cancer, neurological disorders, and inflammatory and autoimmune diseases.

SUPPLEMENTARY INFORMATION

Refer to Web version on PubMed Central for supplementary material.

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Components of the cytoskeleton and their cellular roles

Actin filaments (~7 nm in diameter) are polar with a fast growing barbed end (also called the plus (+) end) that is involved in assembly and a slow growing pointed end (also called the minus (–) end) that is involved in disassembly 26 (see the figure, part a). Actin filament networks are important for subcellular structures, the performance of numerous cellular functions

related to cell shape and movement, and membrane trafficking events such as phagocytosis and autophagy 26,148 .

Microtubules (~25 nm in diameter) are polar with one end (the + end) that grows faster than the other (the – end) 27 (see the figure, part b). Microtubules can assemble and disassemble at the + end through a process known as dynamic instability 27 . The rapid disassembly of microtubules is referred to as catastrophe, and the rapid growth of microtubules is referred to as rescue 27 . These dynamic properties enable cells to rapidly organize microtubules, divide, establish cell shape, facilitate cell motility and vesicle trafficking, and deliver signals for bacterial sensing and host cell death 27,149 .

Intermediate filaments — such as keratins, vimentin and lamins — are a heterogeneous protein family (with ~70 members in humans) that have different functions and cell-specific expression patterns 28 . Intermediate filaments assemble from unit length filaments (ULFs) into nonpolar filaments of ~11 nm in diameter, and they exhibit less dynamic behaviour than actin filaments or microtubules 28 (see the figure, part c). Intermediate filaments are motile and flexible structures that provide mechanoprotection and regulate a wide variety of cellular growth and stress-related signalling events including pathogen sensing and autophagy 28,131 .

Septins are GTP-binding proteins that associate with cell membranes, actin filaments and microtubules 29 (see the figure, part d). Septin subunits are classified into different homology groups on the basis of sequence similarity (see the figure; depicted by different shades of red). Subunits from different homology groups interact through their GTP-binding domain (called the G interface) and amino-terminal and carboxy-terminal regions (called the NC interface). In this way, septins form hetero-oligomeric complexes and rods (32–40 nm in length) that assemble into nonpolar filaments and higher-order assemblies, such as rings and cage-like structures (~0.6 µm in diameter) 29 . By functioning as scaffolds and diffusion barriers, septins have key roles in numerous biological processes, including cell division, cellular compartmentalization and host–pathogen interactions 29,150 .

Modification of the cytoskeleton by bacterial pathogens Several intracellular bacterial pathogens modify actin dynamics to invade non-phagocytic cells 151 (see the figure, part a). *Listeria monocytogenes*, *Yersinia pestis* and *Yersinia pseudotuberculosis* use the zipper mechanism of invasion 152 . Zippering occurs when bacterial surface proteins bind host cell receptors and thereby induce actin-dependent membrane rearrangement apposed tightly around the invading bacterium. Other invasive bacteria, such as *Shigella flexneri* and *Salmonella enterica* subsp. *enterica* serovar Typhimurium, use the trigger mechanism of invasion 153 . Triggering occurs when the pathogen injects type III secretion system (T3SS) effector proteins across the host membrane, which induces actin-rich membrane ruffles that mediate macropinocytosis to engulf the bacterium. Surface-adherent pathogens, such as enteropathogenic or enterohaemorrhagic *Escherichia coli* (EPEC or EHEC, respectively), use their T3SS to secrete a transmembrane receptor into the host membrane to stimulate actin polymerization and generate cellular extensions called pedestals 154 . Pedestal formation occurs when actin is polymerized beneath the host cell membrane to which the bacterium is attached, pushing the attached bacterium upward. Other extracellular bacteria, such as *Clostridium* spp., release toxins that disorganize actin to reduce immune cell migration and break down epithelial cell barriers 76 . In all cases, bacteria modify host actin dynamics by manipulating RHO GTPases, such as RHO, RAC1 and cell division cycle 42 (CDC42) (REF. 155). The RHO GTPases act as molecular switches in controlling actin dynamics by regulating the actin-related protein 2/3 (ARP2/3) complex 26 . Various bacteria also modulate the GTPase cycle of proteins involved in membrane and vesicle trafficking — for example, those belonging to the RAS-related proteins in brain (RAB) and ADP ribosylation factor (ARF) families — by using effectors or toxins to modify the activity of host regulators 156 .

Actin polymerization is manipulated by several cytosolic pathogens — such as *L. monocytogenes*, *S. flexneri*, *Rickettsia* spp. in the spotted fever group (*R. conorii* and *R. parkeri*), *Burkholderia* spp. (*B. thailandensis*, *B. pseudomallei* and *B. mallei*) and *Mycobacterium marinum* — which escape from the phagosome and use actin tails to move within and between cells 157–159 (see the figure, part b). Many of these bacteria

promote actin polymerization by exploiting the ARP2/3 complex in different ways. For example, *L. monocytogenes* ActA and *R. conorii* RickA are mimics of the Wiskott–Aldrich syndrome protein (WASP) family, whereas *S. flexneri* IcsA and *M. marinum* (via an unknown effector dependent on the ESAT-6 secretion system 1 (ESX-1)) directly recruit WASP family proteins to promote ARP2/3 activity 157 . How the other actin-binding proteins — including septins, which can form rings around bacterial actin tails 96 — regulate actin-based motility is relatively unknown.

Other intracellular bacteria manipulate the cytoskeleton to establish a niche inside a vacuolar compartment 160 (see the figure, part c). For example, *S. Typhimurium* resides in the *Salmonella*-containing vacuole (SCV), which is a modified phagocytic compartment. SCV integrity is closely linked to microtubules and microtubule motors (for example, dynein and kinesin 1) 102 . *S. Typhimurium* replication coincides with the formation of *Salmonella*-induced filaments (SIFs) extending from the SCV along microtubules, a process that requires SifA and the host protein SifA kinesin 1- interacting protein (SKIP; also known as SNW1) 102 . Intermediate filament proteins also contribute to the establishment of replicative niches. For example, *Chlamydia trachomatis* rearranges vimentin, cytokeratin 8 and cytokeratin 18 to form an inclusion for their replication 108 . The integrity of the *C. trachomatis* inclusion also depends on the bacterial inclusion membrane protein InaC, F-actin assembly, and the recruitment of ARF and 14-3-3 proteins 161 .

Figure 1.

Roles for the cytoskeleton in innate immunity and cell-autonomous restriction of bacterial infection. Actin, microtubules, intermediate filaments and septins have key roles in the detection of bacterial pathogens and the mobilization of antibacterial responses. **a** | Actin assemblies at the plasma membrane provide rigidity to the cell, act as a scaffold to restrain membrane-bound proteins (for example, receptors) and enable endocytosis of receptors, such as Toll-like receptors (TLRs) 77,78,82–84,86. **b** | Cytoplasmic bacteria that polymerize actin, including *Shigella flexneri* and *Mycobacterium marinum*, can be trapped in septin cages, which have been shown to restrict actin-based motility and bacterial dissemination 96,97. **c** | Microtubules and microtubule motors traffic cargo, such as autophagosomes, inside the cell 27,149. There are different types of microtubule motors: plus (+) end motors and minus (–) end motors are classified depending on the direction in which they travel along microtubules 27,149. Autophagosomes move from peripheral locations in the cell to

the microtubule organizing centre (MTOC), a major site of microtubule nucleation, where lysosomes are concentrated for autophagosome–lysosome fusion 94,95,163 . d | Extracellular F-actin on necrotic cells can act as a danger signal 67,68 . Necrosis leads to the loss of membrane integrity, exposing the actin cytoskeleton, and the exposed F-actin acts as a ligand for the C-type lectin domain family 9 member A (CLEC9A) 67,68 . e | Cytoskeleton rearrangements caused by bacterial invasion or toxins are recognized by sensors, such as the cytosolic receptors nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and pyrin, which leads to the activation of innate immune signalling pathways 31,33–35,44 . f | The cytoskeleton can function as a scaffold for autophagy inhibition 94,95 ; intermediate filaments suppress autophagy by forming a complex with proteins that are crucial for autophagy initiation (for example, beclin 1) 107 .

Figure 2.

Detection of bacteria-induced cytoskeletal changes by NOD-like receptors and pyrin. a | Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) detects invasion by *Salmonella enterica* subsp. *enterica* serovar Typhimurium and SopE-induced actin polymerization and membrane ruffling 31 . SopE activates the GTPases RAC1 and cell division cycle 42 (CDC42) via its guanine nucleotide exchange factor (GEF) activity 31 . NOD1 forms multiprotein complexes with SopE, HSP90, and active RAC1 or CDC42 (indicated by an asterisk) 31 . After detection of peptidoglycan (PG) fragments containing d-

glutamyl-meso-diaminopimelic acid (iE-DAP) ligands, activated RAC1 is essential for NOD1 signalling 31 . Downstream signalling uses receptor-interacting protein 2 (RIP2)-dependent activation of the transcription factors nuclear factor- κ B (NF- κ B) and activator protein 1 (AP-1). Constitutively active forms of RHOA (not depicted), RAC1 and CDC42 can also be sensed by NOD1 to activate RIP2-dependent transcriptional changes 31 . b | NOD1 detects *Shigella flexneri* invasion. *S. flexneri* iE-DAP and the effector proteins IpgB2 and OspB are detected by NOD1, and this depends on actin dynamics controlled by its interacting partners GEF-H1 (which is a RHOA GEF) and Slingshot homologue 1 (SSH1; a cofilin phosphatase) 34,35 . GEF-H1 activates RHOA-dependent actin dynamics and downstream effectors such as RHO-associated protein kinase 1 (ROCK1) 34 . SSH1 dephosphorylates cofilin, which increases its actin depolymerization activity 35 . Downstream NF- κ B activation by iE-DAP involves RHOA and RIP2, whereas NF- κ B activation by OspB and IpGB2 requires RHOA and ROCK1. c | Alterations of RHOA, RHOB or RHOC by bacterial toxins (indicated by asterisks) are detected by pyrin 44 . A number of bacterial toxins with different biochemical activities covalently modify and inhibit the indicated RHO GTPases (for more details see TABLE 1). Toxin-induced inhibition of RHO GTPases results in altered balance between F-actin and G-actin and cytoskeleton dysfunction, which is sensed by pyrin. The assembly of inflammasomes by pyrin activates caspase 1, which processes interleukin-1 β (IL-1 β) and IL-18, and triggers cell death by pyroptosis. ASC is an adaptor that allows the recruitment and activation of caspase 1 by pyrin. d | Actin, microtubules and vimentin intermediate filaments in NOD-, LRR- and pyrin domain-containing 3 (NLRP3) activation. Actin is required for phagocytosis of particulate agonists, such as monosodium uric acid (MSU) crystals and calcium pyrophosphate dehydrate (CPPD), to activate NLRP3 (REF. 58). The MEC17 acetylase and the sirtuin 2 (SIRT2) deacetylase modulate the levels of acetylated α -tubulin in cells 57 . Acetylation of α -tubulin favours trafficking of mitochondria containing the adaptor protein ASC to endoplasmic reticulum (ER) sites containing NLRP3 and promotes inflammasome assembly 57 . Vimentin intermediate filaments interact with NLRP3 and promote its activation by soluble agonists (for example, ATP-P2X7 receptor signalling) as well as particulate agonists (such as MSU crystals and asbestos) 59 .

Figure 3.

The roles of the cytoskeleton in innate immune signalling. **a** | F-actin functions as a danger signal. Surface-exposed actin on necrotic cells is detected by antigen-presenting CD8 α^+ dendritic cells (DCs) 67–69. Specific DC subsets express C-type lectin domain family 9 member A (CLEC9A), which is a receptor for actin. Signalling via spleen tyrosine kinase (SYK) promotes antigen cross-presentation and does not activate transcriptional responses. **b** | Dectin 1 signalling and phagocytosis. Signalling by dectin 1 — for example, during the detection of β -glucan particles — activates SYK-dependent and CARD9-, BCL-10- and

MALT1-containing complex (CBM complex)-dependent pro-inflammatory gene expression 78 . Inhibition of actin dynamics by cytochalasin D results in frustrated phagocytosis and heightened pro-inflammatory signalling (indicated by thicker arrows). c | The role of the cytoskeleton in endocytosis of Toll-like receptor 4 (TLR4) and maturation of TLR9-containing endosomes. TLR4 endocytosis determines signalling via myeloid differentiation primary response protein 88 (MYD88) on the plasma membrane and via TIR domain-containing adaptor protein inducing IFN β (TRIF) on endosomes. The two adaptor proteins MYD88 and TRIF induce different transcriptional programmes, as depicted 4 . Actin dynamics control the maturation of TLR9-containing endosomes into signalling-competent lysosome-like vesicles 82 . Dedicator of cytokinesis 2 (DOCK2) and the GTPase RAC1 are involved in modulating actin to promote receptor trafficking and TLR9-dependent induction of type I interferons (IFNs) 83,84 . AP-1, activator protein 1; IKK, I κ B kinase; IRAK, interleukin-1 receptor-associated kinase; IRF, IFN-regulatory factor; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor- κ B; TAK1, TGF β -activated kinase 1; TBK1, TANK-binding kinase 1; TNF, tumour necrosis factor; TRAF6, TNF receptor-associated factor 6.

Figure 4.

Cytoskeletal dynamics and cell-autonomous control of bacterial infection. a | The *Listeria monocytogenes* protein ActA — which recruits the actin-related protein 2/3 (ARP2/3) complex and polymerizes actin tails — coats the bacterium and prevents recognition by ubiquitin, p62, nuclear dot protein 52 (NDP52) and microtubule-associated protein 1 light chain 3 (LC3) 97,101. Thus, *L. monocytogenes* avoids autophagy and septin caging by expressing ActA 97,101 (see Supplementary information S1 (table)). Alternatively, *Shigella flexneri* uses IcsA to recruit neuronal Wiskott–Aldrich syndrome protein (N-WASP) and

ARP2/3, and polymerize actin tails 30 . IcsA-mediated actin polymerization and recognition by autophagy critical components (ubiquitin, p62, NDP52, ATG5 and LC3) are essential both for septin cage assembly and for targeting to autophagy 96–98 . To prevent recognition of IcsA by the host cell, *S. flexneri* can express IcsB 96–98 (see Supplementary information S1 (table)). b | Roles of caspase 4 in actin dynamics. Caspase 4 interacts with the actin-depolymerizing WDR1–cofilin complex through its amino-terminal caspase activation and recruitment domain (CARD), and with flightless 1 via its carboxy-terminal catalytic p30 domain 118,119 . Caspase 4 controls actin depolymerization and cellular migration and is present at the leading edge in macrophages 118,119 . Restriction of intracellular growth of *Legionella pneumophila* requires its delivery to lysosomes, which is an actin-dependent process controlled by caspase 4 (REF. 122). c | Actin dynamics controlled by the NOD-, LRR- and CARD-containing 4 (NLRC4) inflammasome. During *Salmonella enterica* subsp. *enterica* serovar Typhimurium infection, NLRC4 is required for actin dynamics around *S. Typhimurium*, the assembly of inflammasome foci containing the adaptor protein ASC and the generation of mitochondrial reactive oxygen species (ROS) to restrict bacterial growth 120 . NLRC4 signalling also leads to caspase 1 activation, maturation of interleukin-1 β (IL-1 β) and IL-18, and pyroptosis.

Table 1
Detection of bacterial manipulation of the cytoskeleton by host proteins

Host sensor	Cytoskeleton target	Bacterial effector(s)	Effector mode of action	Refs
NOD1	RAC1, CDC42	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar Typhimurium SopE	GEF	31 34, 35
	RHOA	<i>Shigella flexneri</i> IpgB2	GEF	65, 66
	N.D.	<i>Shigella flexneri</i> OspB	N.D.	65, 66
PYRIN	RHOA, RHOB, RHOC	<i>Clostridium difficile</i> TcdB	Glucosylation (T37 in RHOA)	44
		<i>Vibrio parahaemolyticus</i> VopS	Adenylylation (T37 in RHOA)	44
		<i>Histophilus somni</i> IbpA	Adenylylation (Y34 in RHOA)	44
		<i>C. botulinum</i> C3 toxin	ADP-ribosylation (N41 RHOA)	44
		<i>Burkholderia cenocepacia</i> unknown toxin	Deamidation (N41 RHOA)	44, 45
	N.D.	<i>Francisella tularensis</i>	N.D.	47

CDC42, cell division cycle 42; GEF, guanine nucleotide exchange factor activity; N.D., not determined; NOD1, nucleotide-binding oligomerization domain-containing protein 1; RAC1, Ras-related substrate of botulinum C3 toxin 1