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Cell-autonomous immunity by IFN-induced GBPs in animals and plants

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Inside host cells, guanylate binding proteins (GBPs) rapidly assemble into large antimicrobial defense complexes that combat a wide variety of bacterial pathogens. These massive nanomachines often completely coat targeted microbes where they act as recruitment platforms for downstream effectors capable of direct bactericidal activity. GBP-containing platforms also serve as sensory hubs to activate inflammasome-driven responses in the mammalian cytosol while in plants like *Arabidopsis*, GBP orthologues may facilitate intranuclear signaling for immunity against invasive phytopathogens. Together, this group of immune GTPases serve as a major defensive repertoire to protect the host cell interior from bacterial colonization across plant and animal kingdoms.

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Introduction

Cellular self-defense is a fundamental trait of all living organisms [1]. In *Bacteria* and *Archaea*, the recent discovery of CRISPR-Cas and other anti-phage systems are beginning to reveal the full range of microbial defense repertoires operating in nature [2••,3], while in viruses analogous immune components such as MIMIVIRE have been identified [4]. These elaborate systems of single-cell defense help protect prokaryotic genomes against foreign DNA invasion by virophages, bacteriophages and plasmids. Eukaryotes have likewise evolved sophisticated nucleic acid-based recognition machinery and effector mechanisms to cope with microbial threats from the outside world [5]. In particular, land plants exhibit a rich antimicrobial armamentarium, some of which are shared with animal species including humans [6].

As one moves toward longer-lived vertebrates, important antimicrobial pathways are often placed under tight regulatory control, for example, by the interferon (IFN) family of cytokines which arose in early gnathostomes [7–9]. IFNs direct the transcriptional and post-translational regulation of hundreds of IFN-stimulated genes (ISGs) [8]. Network analysis suggests ISGs conform to a modular design where proteins with common functions are co-opted to defend against major pathogen classes [10••]. Among the ISGs recently found to serve as protective hubs are a novel family of immune GTPases — the 65–73 kDa guanylate binding proteins (GBPs) [11]. A growing body of work has implicated the GBPs in cell-autonomous immunity against a broad list of facultative and obligate intracellular pathogens [12,13•].

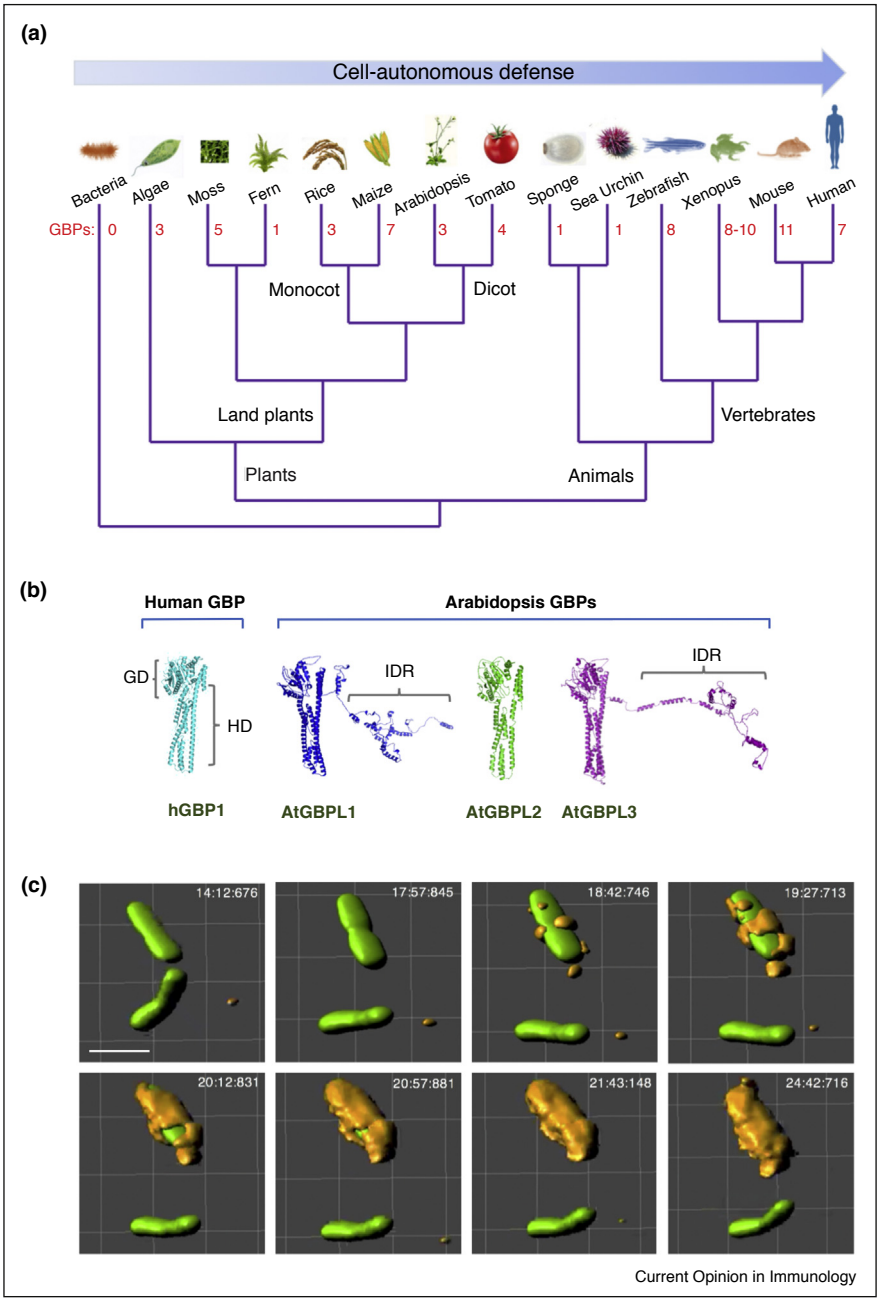
This review briefly introduces the GBPs with an emphasis on their host defense activities against bacterial pathogens at the single cell level. Recent advances in GBP-mediated activation of inflammasomes and their potential intranuclear role in plant immunity will also be discussed.

Eukaryotic evolution of GBPs

Initial phylogenetic mining of *GBP*-like genes across evolution via Hidden Markov modeling retrieved 132 intact ORFs belonging to 32 taxa [14]; this group has recently been expanded to >200 orthologues including genetically tractable plant species like *Arabidopsis thaliana*, *Oryza sativa* and *Solanum lycopersicum* [13•] (Figure 1a). Existence of these additional orthologues suggests primordial defense activities by GBPs are still operative in organisms that lack motile immune cells and a system of IFN-inducible immunity.

In amphibians, jawed fish, marsupials, and mammals, the GBPs have expanded within euchromatic clusters. For example, 7 *GBP* genes and 1 pseudogene reside in a single cluster on human chromosome 1q22.2; close orthologues are present in many anthropomorphic primates [13•,14,15] (Figure 1a). Familial GBP clusters likewise predominate in mice, rats, and opossums with 11, 8 and 6 intact genes, respectively; these genes are distributed across one or two compact chromosomal regions [13•,15]. In zebrafish and

Figure 1



Evolution of GBPs in plants and animals. **(a)** Simple unrooted phylogram of GBPs and GBP-like (GBPL) orthologues across selected animals and plants. Number of family members uncovered from genome NCBI BLAST searches and Plaza (https://bioinformatics.psb.ugent.be/plaza/) depicted in red font. **(b)** 3D protein structure prediction (I-TASSER) of *Arabidopsis thaliana* GBPLs versus crystallized hGBP1 (PDB 1F5N). In addition to the catalytic GTPase domain (GD) and C-terminal helical domain (HD) found in humans, some plant GBPLs also possess long C-terminal extensions that contain intrinsically disordered regions (IDRs). **(c)** Antibacterial activities of GBPs operate in animals and plants. In IFN-γ-induced human HeLa epithelial cells, GBP1 completely coats *Salmonella typhimurium* or its *Salmonella*-containing vacuole as shown by live wide-field imaging. 3D-rendered views constructed using Imaris software. Scale bar, 2 μm.

frogs, between 8–10 *GBP* genes are located together on 3 small genomic islands, suggesting duplicative events help generate familial diversity across multiple GBP-expressing species [13•,15].

GBP expression and enzymology

Transcriptional induction of these genes in vertebrates typically requires signaling via IFNs type I (IFN-α/β), II (IFN-γ) and III (IFN-λ) depending on cell or tissue type

[13•]. Constitutive IFN-β signaling has also been reported to maintain low tonic levels of GBP expression in cultured mouse macrophages [16•]. Other pro-inflammatory cytokines like IL-1α, IL-1β or TNF-α can induce human GBP expression within tissue endothelium as well as colonic epithelium, albeit at much lower levels than IFNs [17]. Indeed, receptors for many of these cytokines are quite ubiquitous, especially the IFN-γ receptor which is found on nearly all nucleated cells [8]. For this reason, human GBPs are expressed in multiple cell lineages. Curated RNASeq and microarray profiles have recently uncovered robust human GBP expression in 83 of 84 cell and tissue types examined [13•]. Thus GBP-driven defense operates both inside and outside of the classical immune system as part of the cell-autonomous response [11].

The mammalian GBP proteins synthesized from these transcripts act as ~65–73 kDa GTPases whereas in plants like *Arabidopsis* several catalytically active GBP-like (GBPL) family members migrate at ~110 kDa due to C-terminal extensions (S. Huang, unpublished observations) (Figure 1b). Most mammalian GBPs harbor a bidomain architecture with an N-terminal GTPase domain and C-terminal helical domain comprising a series of amphipathic helices based on crystallography studies [17,18]. Human and mouse GBPs share 40–98% amino acid identity across these domains; plant GBPLs share ~20–32% with human GBPs. Each half typically contributes to nucleotide-dependent self-assembly as seen for other IFN-induced GTPases which possess dynamin-like properties to form large homotypic complexes [19]. In human and mouse GBP1, GBP2 and GBP5 the C-terminus contains CaaX motifs for isoprenylation; the latter facilitates membrane anchorage to the endoplasmic reticulum as well as intermediates of the endolysosomal pathway under steady-state conditions [20]. Other family members largely reside in the cytosol and may partition with these GBP membrane-anchored partners as heterotypic complexes to target bacterial pathogens when cells are infected [11,13•].

Human and mouse GBPs bind GTP, GDP, and GMP with equimolar affinity [11]; the physiological importance of this unique profile is unknown although these guanosine nucleotides may drive oligomerization or be the produced as a result of oligomeric self-assembly. The latter leads to supramolecular GBP structures which can completely coat cytosolically exposed bacteria or parasites inside host cells [21–23,24••] (Figure 1c). Remarkably, these giant nanomachines may reach 6000 subunits [23] that can serve as a recruitment platform for antimicrobial partners involved in oxidative or inflammasome-mediated defense [14,22,25] (Figure 2a,b). The latter is reminiscent of other innate immune signaling platforms that form supramolecular organizing centers (SMOCs) [26]. In addition, GBP coats may exert mechanoenzyme activity to disrupt the outer membrane of bacteria [27,28] or disable the host actin cytoskeleton to immobilize pathogens [24••].

GBP immunity to bacteria: early discoveries

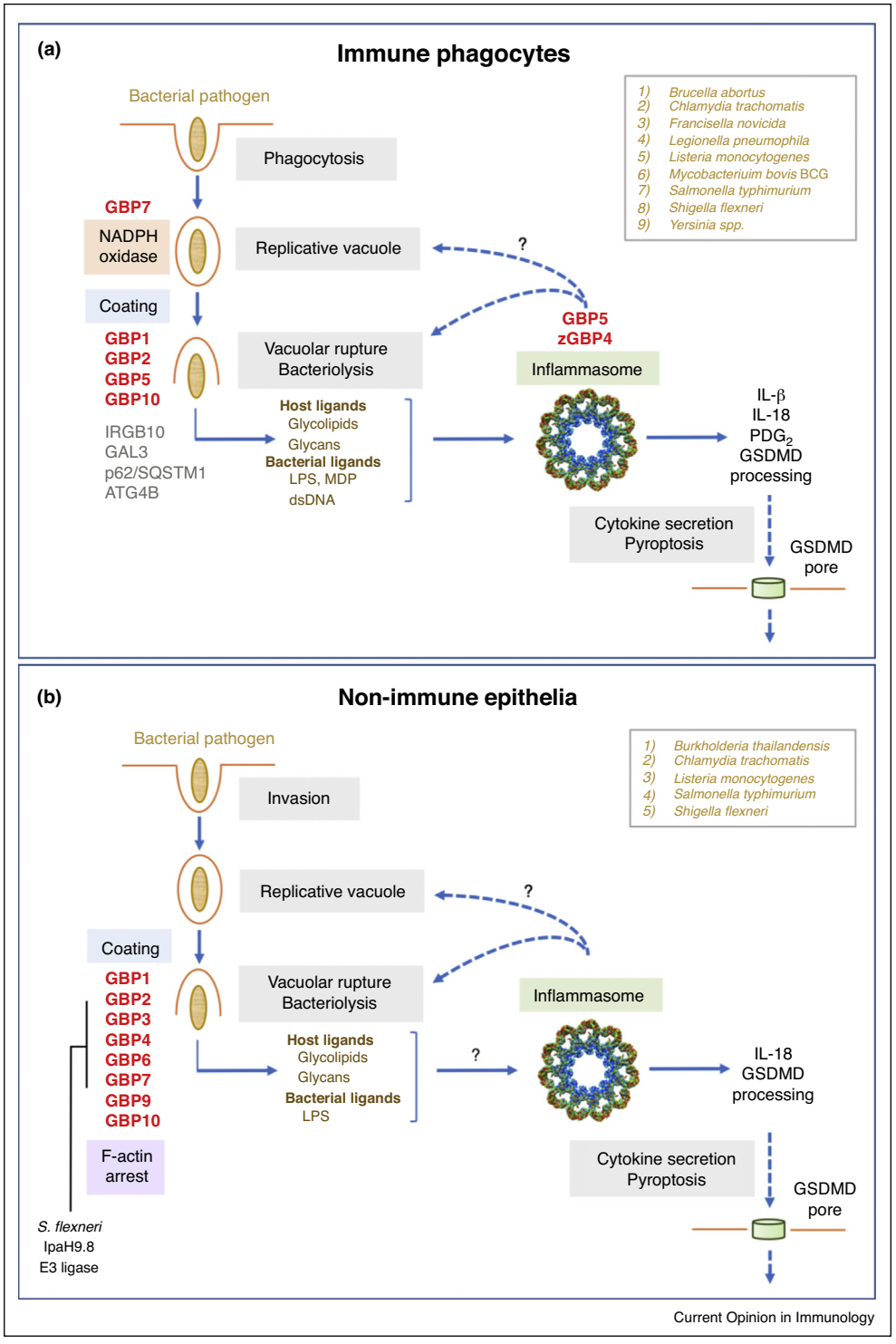
Cell-autonomous defense against intracellular bacteria has been the most studied area of GBP immunity to date. In 2011, the first Gbp-deficient ($Gbp1^{-/-}$) mice were reported (Table 1); their phenotype showed impaired antibacterial activity against *Listeria monocytogenes* and *Mycobacterium bovis* BCG [22], the latter of which causes disseminated mycobacteriosis in IFNGR-deficient patients [29]. Gbp1 along with three other family members – Gbp6, Gbp7, and Gbp10 – conferred host resistance as shown by loss-of-function screens across the complete Gbp family in IFN-γ-activated macrophages. The following year, newly generated Gbp5-deficient ($Gbp5^{-/-}$) mice revealed diminished inflammasome responses to *Listeria* and *Salmonella enterica Typhimurium* [14]. In contrast, $Gbp2^{-/-}$ mice were protected against *Listeria* but susceptible to the apicomplexan parasite, *Toxoplasma gondii* [30]. These early genetic studies formally established non-redundant roles for GBPs against bacterial pathogens *in vitro* and *in vivo* (Table 1). They also uncovered interacting partners, protein domain relationships, and trafficking behavior that laid the foundation for recent insights into how the GBPs might operate.

For example, Gbp1, Gbp7 and Gbp10 all trafficked to *L. monocytogenes* and *M. bovis* within 30–120 min of uptake; GTPase and isoprenylation mutants blocking GBP relocation also interfered with antimicrobial activity [22]. Numerous reports have now extended this model to other bacterial species including *Chlamydia trachomatis*, *Francisella novicida*, *Legionella pneumophila*, *Shigella flexneri*, *Yersinia pseudotuberculosis* and *Brucella abortus* [16•,24••,25,27,31–38] (Figure 2a,b). Hence targeting to bacteria appears central for cell-autonomous immunity by many GBPs. The consequences for host defense are outlined below.

Bacterial targeting by GBPs: causes and consequences

What triggers GBP trafficking to intracellular bacteria and what are the downstream consequences? (Figure 2a,b) Pathogens like Gram-positive *L. monocytogenes* and Gram-negative *S. flexneri* or *F. novicida* escape their vacuole shortly after uptake to replicate in the host cell cytosol, whereas *S. typhimurium* enters the cytosol at later times in smaller numbers. Nonetheless, these cytosolic subpopulations contribute significantly to overall bacterial replication and are targeted by GBPs [21,24••,32,35••] (Figure 1c). In contrast, *M. bovis* BCG lacks a chromosomal region encoding part of the bacterial type VII secretion (T7SS) apparatus needed for escape and remains trapped inside a phagocytic compartment unless this vacuole is disrupted [8,11]. *Chlamydia trachomatis*

Figure 2



Antibacterial functions of GBPs in immune and non-immune cells. Specific GBP family members involved in intrinsic host defense in **(a)** immune and **(b)** non-immune cells. Specific assembly of the NADPH oxidase, inflammasome components and IRGB10, as well as the block in F-actin polymerization are depicted. Inflammasome assembly may take place directly on targeted bacteria as indicated by question marks. Likewise, in epithelia it is unknown which intraluminal host ligands are released to help drive downstream events, as again shown via a question mark. GBP-interacting partners Galectin-3, p62/SQSTM1 and ATG4B are in grey font with bacterial species targeted by GBP-mediated defense activities boxed in the upper right corner. The *Shigella* E3 ligase IpaH9.8 responsible for ubiquitinating GBPs followed by proteasomal degradation is also shown. zGBP4, zebrafish GBP4.

Table 1

Bacterial immunity phenotypes in GBP-deficient mice

GBP Knockout	Bacterial Challenge	Phenotype	Reference
$Gbp1^{-/-}$	*L. monocytogenes*	Susceptible to orogastric infection	[22]
	M. bovis BCG	Susceptible to i.v. infection	[22]
$Gbp2^{-/-}$	*L. monocytogenes*	Resistant to i.p. infection	[30]
	F. novicida	Susceptible to s.c. infection and reduced serum IL-18	[28]
	OMV i.p. challenge	Resistant to endotoxemia after poly I:C priming. Reduced serum IL-1β plus IL-18	[25,51]
$Gbp5^{-/-}$	*L. monocytogenes*	Susceptible to orogastric infection and insensitive to the caspase-1 inhibitor z-YVAD-FMK	[14]
	LPS i.p.challenge	Reduced serum IL-1β plus IL-18 and reduced active caspase-1 in splenic macrophages	[14]
	MDP i.p.challenge	Impaired peritonitis and reduced active caspase-1 in peritoneal neutrophils	[14]
$Gbp^{chr3-/-}$	*F. novicida*	Susceptible to s.c. infection and reduced serum IL-18	[28]
	S. flexneri	Susceptible to ΔipaH9.8 mutant administered i.v. and i.p.	[47]
	L. pneumophila	Susceptible to cytosolic ΔsdhA *Lpn* administered oropharangeally	[53,35••,16•]
	OMV i.p. challenge	Resistant to endotoxemia after poly I:C priming. Reduced serum IL-1β plus IL-18	[51,25]
	LPS i.p. challenge	Resistant to endotoxemia after poly I:C priming. Reduced serum IL-1β plus IL-18	[25]

i.v., intravenous; i.p., intraperitoneal; s.c. subcutaneous; OMV, Gram-negative outer membrane vesicles; MDP, muramyl dipeptide.

likewise resides within a reticulate structure termed the inclusion body [34]. Both vacuolar species also recruit GBPs. Thus, conserved microbial structures belonging to Gram-positive, Gram-negative and Actinobacteria may solicit these immune GTPases once they gain access to the cytosol after vacuolar damage. Alternatively, 'altered self' ligands in the form of liberated intraluminal host ligands could also serve as proxies of infection [13•,21].

Indeed, sterile lysosomotropic agents cause human GBP1 to relocate to damaged membranes even in the absence of infection [21], suggesting components of the endo-lysosomal pathway can mobilize GBPs. Most GBP-restricted bacteria intersect this pathway where microbial pore-forming toxins and type III (T3SS) or IV (T4SS) secretion systems can cause membrane rupture to release ligands for detection [16•,21,32,38]. Among the likely culprits are intraluminal glycolipids or host glycans normally excluded from the host cell cytosol [21,32] (Figure 2a,b).

GBP recruitment, oligomerization and coating of bacteria provide platforms to assemble effector complexes [11]. Gbp7 partner interaction screens retrieved endogenous $gp91^{phox}$ and $p22^{phox}$ comprising the cytochrome b_{558} heterodimeric membrane (Nox2) of the phagocyte (NADPH) oxidase involved in superoxide (O_2^-) production for antimicrobial defense. Gbp7 promoted targeting and assembly of the Nox2 holoenzyme on intracellular bacteria [22] (Figure 2a). In humans, autosomal mutations in NADPH oxidase components give rise to chronic granulomatous disease (CGD); the latter is characterized by lowered oxidant responses and recurrent infections by catalase-positive bacteria including *Listeria* and *Mycobacterium bovis* BCG [39,40]. Functional silencing of Gbp7 likewise diminished the IFN-γ-induced oxidative burst and NADPH oxidase assembly on bacterial compartments; both defects rendered macrophages more susceptible to these two pathogens [22].

Gbp1 interaction screens retrieved p62/Sqstm1 and the cysteine protease, Atg4b, involved in autophagosomal membrane closure [22]. Gbp1 and p62/Sqstm1 convergence on cytosolic bacteria may involve Galectin-3 that serves as a marker for damaged membranes along with ubiquitination and can also physically complex with Gbp2 [32] (Figure 2a). Neither p62 nor Galectin-3 is obligate, however, for GBP recruitment to escaped *L. monocytogenes*, *S. typhimurium* or *L. pneumophila* in IFN-γ-activated mouse or human cells [21,33]. Hence, they may act to stabilize rather than solicit the GBP coat or eventually help ubiquitinate components to turn off signaling as seen recently for cGAS and the NLRP4 signalosome [41,42].

This massive coat can incorporate multiple GBP family members depending on the pathogen encountered [22–23,24••,35••]. *L. monocytogenes* and *M. bovis* recruit Gbp1, Gbp6, Gbp7, and Gbp10, whereas *F. novicida* solicits Gbp2 and Gbp5 and *C. trachomatis* potentially 7 different Gbps in IFN-γ-activated mouse macrophages and fibroblasts, respectively [22,26,27,36]. Within human epithelia *S. typhimurium*, *S. flexneri* and *B. thailandensis* are targeted by GBP1, GBP2 and GBP4 in a hierarchical manner [21,24••,35••,36,43] (Figure 2b). Why specific family members engage different pathogens is presently

unknown but loss-of-function analysis reinforces the collective contributions by individual GBPs.

In addition to providing a platform for recruiting antimicrobial effectors, polyvalent GBP coating could directly damage bacterial membranes or restrict motility. Loss of outer membrane LPS staining is observed when Gram-negative *S. typhimurium* and *F. novicida* become decorated with Gbp2 or Gbp5 in IFN-γ-stimulated mouse macrophages [27,28,37]. Here, bacterial outer membrane mutants suggest the LPS lipid A moiety may stabilize GBPs on the microbial surface once they are recruited [25]. Subsequent LPS loss could result from GBP mechanoenzyme activity like other dynamin superfamily members that vesiculate membranes [20]. In IFN-γ-activated human epithelia, complete encapsulation of *S. flexneri* by human GBP1, GBP2, GBP3, and GBP4 is also posited to prevent actin-based motility and cell-to-cell spread [24••]. *S. flexneri* expresses an E3 ubiquitin ligase, IpaH9.8, that ubiquitinates GBPs for proteasomal degradation in an effort to escape cell-autonomous immunity [24••,35••,43]. This co-evolutionary arms race underscores the importance of GBPs in placing selective pressure on targeted pathogens to invent mechanisms for immune evasion [44].

GBP-driven inflammasome immunity to bacterial pathogens

Liberation of bacterial products by GBP-mediated vacuolar or microbial cell surface disruption activates cytosolic innate immune pathways including the multiprotein complex termed the inflammasome [12]. In fact, modular similarities between GBPs and the core inflammasome machinery arose from evolutionary multidomain profiling across numerous taxa [12,14]. Here teleost GBPs contained caspase activation and recruitment domains (CARDs) similar to those in human NLRP1, ASC, and Caspase-4 [13•,14]. It suggested GBPs lacking these CARDs and the inflammasome proteins harboring them could still maintain a relationship in mammals despite these domains now being distributed on separate proteins [12].

Such a relationship was shown by stable loss-of-function experiments in IFN-γ-activated human monocytes and mouse macrophages along with Gbp5$^{-/-}$ mice challenged with inflammasome agonists *in vitro* and *in vivo* [14]. GBP5 promoted canonical NLRP3 inflammasome activation by *Listeria*, *Salmonella*, and bacterial products such as LPS and muramyl-dipeptide which has been extended to *Yersinia* and possibly *T. gondii* infections [14,45,46] (Table 1). These effects were evident in IFN-γ-treated but not type I IFN-treated cells [14,22,27–28,37], suggesting GBP5 may enlist other IFN-γ-inducible proteins to help confer its effects or need to be robustly expressed in order to participate in the canonical NLRP3 pathway [12–14]. Gbp5 along with another family member, Gbp2, has also been found to aid the canonical AIM2 inflammasome by helping release dsDNA from damaged *F. novicida* [27,28]; in mouse macrophages, this release enlists a 47 kDa Immune GTPase, Irgb10, to help disrupt the bacterial cell wall [47] (Figure 2a). Furthermore, chromosomal deletion of Gbp2, Gbp5 and potentially other family members in the mouse chromosome 3H1 cluster (*Gbp1* [*Gbp2a*], *Gbp2*, *Gbp3*, *Gbp5*, *Gbp7*) has established a role not only in the AIM2 inflammasome but also the caspase-11-dependent non-canonical inflammasome pathway depending on the ligand or pathogen encountered [16•,25,37,38,48–53] (Table 1). Diminished cytokine secretion and pyroptosis in Gbp knockout mice render them susceptible to infection.

Whether this latter role extends to the non-canonical pathway in humans is currently unknown. Evolutionary CARD domain similarities in human caspase-4 and zebrafish GBPs suggest functional interactions between human GBPs and non-canonical caspases is possible [13•,14]. The zebrafish GBP4 CARD engages an inflammasome adaptor protein, ASC, in order to assemble the Nlrc4 inflammasome complex for prostaglandin D2 release in response to *Salmonella* infection [54]. Hence these GBP-fused CARDs are functional. Likewise, GBP5 interacts with the pyrin domain of human NLRP3 for inflammasome complex assembly and interleukin-1 beta (IL-1β) release in human cells [14]. It will be interesting to determine if GBP-assisted inflammasome assembly and caspase activation takes place directly on cytosolically exposed bacteria, providing a physical link with the GBP trafficking model that initiates pyroptosis to remove infected cells [22,37].

GBP cell-autonomous defense in plants

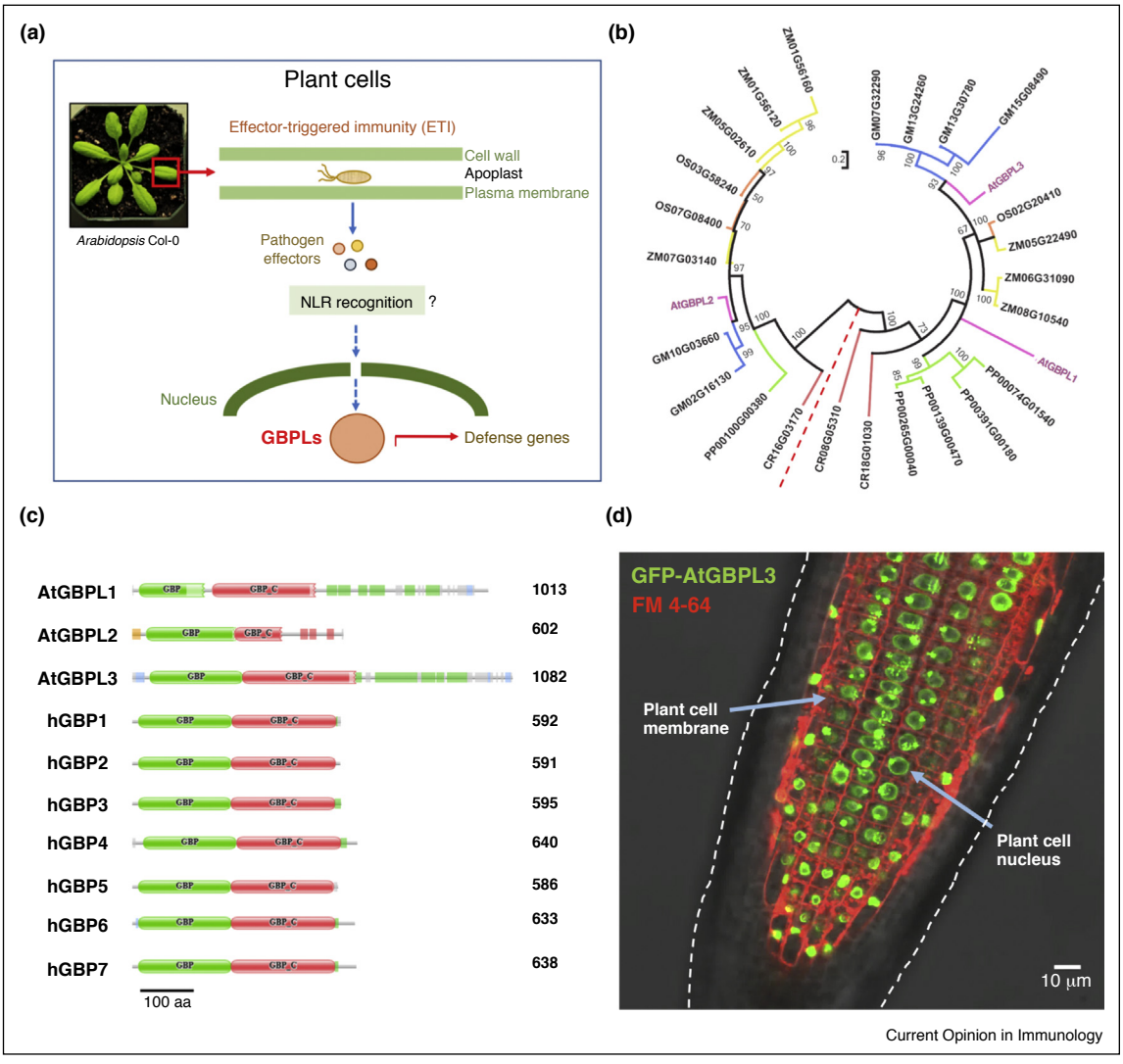
Conceptual parallels for GBP-mediated immunity in animals may apply to plants as well. As sessile hosts devoid of migratory immune cells, plants rely solely on innate immunity to combat infection by phytopathogens [55,56]. Many of these cell-autonomous reactions are polarized beneath microbial contact sites at the cuticle periphery and involve physical interference by *de novo* callose deposition and cell wall biosynthesis and strengthening [57]. Such a cell wall remodeling constitutes a major component of structural defense.

Beyond physical barriers, however, plants also deploy tiered immune systems with an immensely rich armamentarium of immune sensors to perceive and control invading bacterial phytopathogens [6,55]. Many of these systems are inducible by hormone-like signals – notably jasmonate and salicylic acid – with similarity to the cytokine repertoire of animals which act locally over short distances to elicit broad defense programs inside host cells. Plant NLRs mobilized by these inducible signals detect pathogen-derived effectors in the host cell cytosol [6]. They monitor perturbations as part of the 'guard' or

'decoy' hypotheses which bears some resemblance to the 'altered self' model for GBPs where host vacuolar structures mis-localized in the cytosol are recognized as an indirect signature of infection [13•,21] (Figure 3a). Following detection, vertebrate GBPs can engage NLRs, ASC, and possibly caspases to mobilize inflammasome responses [13•,14,54]. Analogous cross-talk may occur in plants as outlined below.

GBP-like (GBPL) orthologues exist in numerous species, particularly land plants from moss and ferns to flowering monocot and dicot hosts (Figure 1a). As many as 7 *GBPL* family members are encoded in the maize (*Zea mays*) genome with lower numbers in other genetically modifiable species, for example, *Arabidopsis thaliana* that contains 3 orthologues, two of which contain integrated domains analogous to NLR integrated domains (Figure 3b,c). Examination of these configurations reveals some *Arabidopsis* GBPLs possess structural maintenance of chromosome (SMC) domains in addition to GTPase and C-terminal helical regions (Figure 3c). Indeed, expression studies reveal certain GBPL members reside within discrete nuclear structures as potential integrators of upstream bacterial sensing by NLRs (S. Huang,

Figure 3



Plant GBPLs in intranuclear immunity. **(a)** Intranuclear immunity to bacterial phytopathogens by *Arabidopsis* GBPLs may operate downstream of cytosolic NLRs as part of the 'guard' hypothesis or effector-triggered immunity (ETI). **(b)** Circular dendogram of GBP-like orthologues in various plant hosts. Abbreviations: ZM, *Zea mays* (maize); OS, *Oryza sativa* (rice); GM, *Glycine max* (soybean); CR, *Chlamydomonas reinhardtii* (algae); PP, *Physcomitrella patens* (moss). *Arabidopsis thaliana* (At) GBPLs are highlighted in color. Branch distances from bootstap of 5000 replicates in maximum likelihood analysis **(c)** Domain architecture of *Arabidopsis* and human GBPs showing reconfigured C-termini for AtGBPL1 and AtGBPL3. **(d)** Nuclear localization of AtGBPL3 shown via stable expression of a GFP-fused construct within *A. thaliana* roots. FM 4–64 visualizes individual plant cell membranes. Overlay of confocal fluorescence with differential interference contrast microscopy.

unpublished observations) (Figure 3a,d). Large number of NLR sensors exist in *Arabidopsis* genomes as well as maize and rice so GBPL proteins likely represent a new intranuclear signaling hub for cell-autonomous defense in many monocot and dicot species. Thus, preliminary evidence suggests the innate immune signaling and sensory network functions of GBPs appear conserved across the plant-animal divide.

Summary and future directions

Profound differences in scale arise during encounters of eukaryotic cells with intracellular bacteria. Here, the pathogen may occupy less than a thousandth of the host cell volume [8]. A major challenge, therefore, is to detect and dispatch GBPs to the site of infection. Recent work indicates structurally diverse bacteria and damaged sterile vacuoles are both targeted by GBPs [13•]. Hence a common host luminal signal rather than microbial ligand probably triggers initial mobilization of these proteins to escaped bacteria [1,21], although cell-wall structures like Gram-negative LPS could help retain GBPs once they reach the bacterial surface [25]. The precise chemical nature of this danger signal constitutes a major question for future research [21,44].

Convergence of GBPs on bacterial pathogens has several fates depending on which GBP family members are involved, what bacterial pathogen is being targeted and the type of host cell being infected [11,13•]. For example, the latter may dictate the choice of effector machinery recruited such as the NADPH oxidase or inflammasome components in IFN-γ-activated macrophages or binding F-actin for motility arrest in epithelial cells [14,22,24••] (Figure 2). Additional interacting partners will further delineate the breadth of GBP antimicrobial activity. Computational approaches similar to that used for NLR biology [58] or IFN-induced proteomics [10••] may be applied to view complex GBP interactions from a systems biology perspective. Indeed, functional GBP-NLR networks are likely to operate across both animals and plants where they could also engage shared SMOCs such as the TRAFasome [26,32,59]. A final frontier involves the discovery of disease-related hypomorphic or nullizygous *GBP* mutations within susceptible individuals; identifying such people will help underscore the importance of these new immune GTPases for human immunity to bacterial infection *in natura*.

Conflict of interest statement

Nothing declared.

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