The origin and evolution of cell-intrinsic antibacterial defenses in eukaryotes

Daniel J. Richter[a] and Tera C. Levin[b]

a. Institut de Biologia Evolutiva (CSIC-Universitat Pompeu Fabra), Barcelona, Catalonia, Spain
b. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States

Corresponding authors: Richter, Daniel J. (daniel.j.richter@gmail.com), Levin, Tera C. (tlevin@fredhutch.org)

Abstract (120 words limit)

To survive in a world dominated by bacteria, eukaryotes have evolved numerous self-defense strategies. While some defenses are recent evolutionary innovations, others are ancient, with roots early in eukaryotic history. With a focus on antibacterial immunity, we highlight the evolution of pattern recognition receptors that detect bacteria, where diverse functional classes have been formed from the repeated use and reuse of a small set of protein domains. Next, we discuss core microbicidal strategies shared across eukaryotes, and how these systems may have been co-opted from ancient cellular mechanisms. We propose that studying antibacterial responses across diverse eukaryotes can reveal novel modes of defense, while highlighting the critical innovations that occurred early in the evolution of our own immune systems.

Short title (50 characters limit): Evolution of antibacterial defenses in eukaryotes

Keywords (1-5): evolution; eukaryotes; antibacterial defense; innate immunity

The unexplored diversity of eukaryotic antibacterial defenses

Since their origin via endosymbiosis more than a billion years ago, eukaryotes have lived in a world dominated by bacteria (1,2). The perpetual struggle to avoid exploitation by bacterial pathogens has shaped molecular strategies for microbial recognition and response across eukaryotic history. Here, we review some of the most ancient cell-intrinsic antibacterial defenses in eukaryotes, which we argue formed the foundation of innate immune systems in groups such as plants and animals (3,4). A more thorough exploration of antibacterial strategies across eukaryotes can illuminate the ancestry of immunity and lead to the discovery of new pathways and mechanisms of antibacterial defense.

Plants, animals, fungi and other highly-studied macroscopic groups represent only a fraction of eukaryotic diversity (Figure 1) (5), while some other eukaryotic lineages have been so little studied that the majority of their species are known through environmental sequencing alone. The disparities between the diversity of studied species and the actual scope of eukaryotic diversity are evident in the biased phylogenetic distribution of formally described genera relative to a sequence-based survey of operational taxonomic units from a global-scale marine dataset (OTUs, a unit of sequence diversity roughly equivalent to genera; Figure 1). This environmental survey from *Tara* Oceans, the largest environmental sequencing project to date, provides what is currently our best window into eukaryotic diversity in the ocean (6). Nevertheless, the *Tara Oceans* dataset is an underestimate of global eukaryotic diversity, as other environments, such as soil, may harbor even higher diversity (7). As shown in Figure 1, most

eukaryotic groups have received little, if any, experimental characterization of antibacterial defenses, so analyses of such defenses currently rely on bioinformatic predictions. Yet even these approaches are limited, as genome-scale data are only available for a small subset of these groups. Specifically, lineages such as Dinophyceae, core syndiniales, Radiolaria and Diplonemea contain comparatively few taxa with formal morphological descriptions or sequenced genomes, yet harbor an enormous diversity waiting to be explored.

In this review, we integrate current research on eukaryotic antibacterial defense systems, together with Pfam protein domain queries of MMETSP data (a large-scale project that sequenced the transcriptomes of several hundred diverse species of marine microbial eukaryotes (8)), to describe cell-intrinsic bacterial recognition and killing mechanisms that are most broadly conserved across eukaryotes. For brevity, we focus on bacteria-proximal steps, rather than intermediates in immune signaling cascades. We consider the following pathogens and mechanisms outside of our scope: (i) gene families restricted to a single eukaryotic lineage (e.g., animal-specific RIG-I-like receptors (9)) (ii) mechanisms that target viruses and selfish elements, including RNAi (10) (see also a review by Zhao and Guo in this issue (11)), (iii) eavesdropping and response to chemical signals produced by bacteria (12,13), and (iv) parasitism of eukaryotes by other eukaryotes (e.g. (14,15)). Instead, we present core antibacterial strategies that are shared across eukaryotes, highlighting those that are deeply conserved and those that have experienced recurrent innovation involving a limited number of functional protein domains. We also propose paths forward to discover unknown defenses in little-studied eukaryotic lineages.

Convergence and conservation in bacterial pattern recognition proteins

A eukaryotic cell's first step in an antibacterial response is to recognize the telltale signs of bacteria, typically via pattern recognition proteins. The evolutionary histories of these proteins fall into two categories: some are found across diverse eukaryotic groups and were therefore likely present in the last eukaryotic common ancestor (LECA), and others evolved after the LECA, within one or more groups independently (Table 1). Among the many gene families in the first category are lectins, a broad class of proteins that has been associated with sugar-based bacterial recognition in multiple eukaryotic groups (16–18). More recently evolved gene families include the cytosolic DNA/cyclic dinucleotide sensors cGAS and STING, which appear to have arisen in eukaryotes only once, in Choanozoa (animals and their closest relatives, Choanoflagellatea) (19) (although cGAS-like proteins exist in bacteria (20)). Here, we focus on the evolutionary histories of three classes of proteins that mediate bacterial recognition and have been well studied in animals and plants: TLRs (Toll-like receptors), RLKs (receptor-like kinases) and NLRs (nucleotide-binding domain, leucine-rich repeat superfamily proteins) (21–23) (Figure 2). These classes can be identified by unique combinations of protein domains, each of which are associated with known functions. Leucine rich repeat (LRR) domains, which are common to all three classes, can recognize molecules produced either by bacteria or by the eukaryotic cell in response to a bacterial attack. Animal TLRs, which sense extracellular bacterial molecules or those within intracellular vacuoles, pair signal recognition via LRR domains with cytoplasmic TIR domains, which transmit the signal inside the cell. RLKs studied in plants pair perception by extracellular LRRs (or a lysin motif, LysM) with intracellular kinase domains (24). NLRs are cytoplasmic recognition proteins that are thought to have evolved convergently in both plants and animals, where they participate in distinct antibacterial defense pathways (25). NLRs commonly found in animals pair NACHT domains with LRRs, while those of plants encode NB-ARC and LRR domains.

Outside of animals and plants, a survey of eukaryotic diversity indicates a surprising number of eukaryotic lineages have proteins with both canonical and novel combinations of LRR, TIR, NACHT and NB-ARC domains (Figure 2). Canonical TLR domain architecture appears to be restricted to Choanozoa

(26). In striking contrast, RLK-like domain architectures are extremely broadly distributed (27), found across every eukaryotic lineage except Apicomplexa. NLR domain pairs (NACHT/LRR and NB-ARC/LRR) are present in multiple evolutionarily distant lineages, although several of the well-studied and functionally characterized NLRs in animals and plants (NOD, NLRP and RPS) (28) appear to be lineage-specific elaborations of ancient and widespread NACHT/LRR or NB-ARC/LRR proteins (23,25) (Figure 2).

Beyond these canonical domain architectures, other eukaryotes have shuffled the domains in their pattern recognition proteins into novel combinations that hint at divergent modes of antibacterial signaling, potentially via more direct routes. For example, animal TLRs initiate downstream kinase signaling cascades via interaction of their TIR domains with intracellular adapter proteins (29). In Choanoflagellatea, there is a TLR-like protein (known as a 'kinase TLR') that contains its own intracellular kinase domain, which may directly initiate a signaling cascade (26) analogous to plant RLKs, which pair extracellular LRR or LysM sensing domains with intracellular kinases (24).

In a parallel example, some animal NLRs include a protein domain (CARD or PYRIN) that functions to recruit caspase proteases, which cleave downstream substrates, generally in the context of programmed cell death (30). Two independently-evolved NLR-like proteins in Diatomeae and in Haptista directly link LRR/NACHT or LRR/NB-ARC domains to a CHAT domain, a caspase-related peptidase domain that may directly perform caspase-like functions (Figure 2). These intriguing cases remain to be experimentally characterized, but may provide important clues as to how immune signaling cascades have been wired and rewired over evolutionary time.

There is also experimental evidence for bacterial recognition and response in lineages with unusual combinations of TLR-like or NLR-like domains. In *Dictyostelium* (Amoebozoa), response to bacterial lipopolysaccharide depends on a protein that combines a TIR-domain with RCC1 and Ankyrin repeat domains (31). Fungi (Nucletmycea) encode a diversity of NLR-like proteins (32) where NACHT and NB-ARC domains are linked to a variety of enzymatic and protein-protein interaction domains, but while fungi have been shown to produce antibacterial compounds in response to bacteria (33), a role for fungal NLRs in this recognition has not been identified. In Choanoflagellatea, there is an NLR-like protein that links LRR and NACHT domains with phospholipase functional domains (PI-PLC-X/PI-PLC-Y). In all of these cases, immune signaling domains have been organized into new contexts relative to well-studied proteins in animals and plants. These examples of evolution by domain shuffling are particularly intriguing, because they may link bacterial sensing to novel downstream signaling cascades.

Given these observations, how might pattern recognition proteins have originated in eukaryotes? Although canonical pattern recognition domain combinations are found in relatively restricted sets of eukaryotic lineages, their core constituent domains (LRR, TIR, Pkinase, NB-ARC, NACHT) were each present in the LECA. This suggests either that TLR-, RLK- and NLR-like domain architectures evolved independently in multiple lineages via domain shuffling, or that these proteins were present in the LECA and subsequently lost numerous times independently. Alternatively, they may have experienced more complex scenarios involving a history of horizontal gene transfer and repeated gene loss. Distinguishing among these alternative scenarios should be possible with increased species sampling and detailed phylogenetic analyses. Overall, these examples illustrate how a relatively small set of individual protein domains appear to have been used and reused as functional building blocks in the evolution of diverse pattern recognition proteins across eukaryotes.

Ancient mechanisms of cell-intrinsic, microbicidal control

Once recognized by the cell, eukaryotes deploy a number of cellular and molecular strategies to kill bacteria. Some conserved mechanisms of cell homeostasis that were present in the LECA can be re-deployed to eliminate bacteria (Table 1). However, because the proteins involved may retain their homeostatic roles, predicting whether a given eukaryote uses these proteins for pathogen defense is often not possible based on sequence analysis alone, and instead requires experimental validation. Nevertheless, we present modes of bacterial killing that appear to be most broadly conserved across eukaryotes.

Eukaryotic species with robust cell walls primarily interact with bacteria extracellularly (34), whereas species with more permeable barriers and/or those that uptake bacteria as food require both intracellular and extracellular killing mechanisms. Intracellular bacteria can be targeted using cellular machinery involved in the degradation and recycling of macromolecules. For example, autophagy, which likely dates back to the LECA (35), allows eukaryotic cells to break down organelles or cytoplasmic contents during periods of cellular damage and stress (36). Autophagy can also encapsulate and eliminate intracellular bacteria, both those residing within host vacuoles and those that have escaped into the cytosol (Figure 3). This process, referred to as “xenophagy”, has been shown to fight bacterial infections within the cells of animals (37) and Amoebozoa (38). Eukaryotes also deploy a number of strategies to restrict microbial growth within the cell. Bacteria internalized via phagocytosis or other uptake pathways are typically sequestered within lysosomes and/or digestive vacuoles (Figure 3) (39). Here, multiple antibacterial strategies are simultaneously deployed to transform vacuoles into toxic, nutrient-poor microenvironments. These strategies include compartment acidification, the generation of reactive oxygen species (ROSs), the activity of proteases, nucleases, and other enzymes to break down macromolecules, and the use of vacuolar transporters to remove nutrients such as iron that could potentially support microbial growth (40). This compartmentalization allows eukaryotes to efficiently kill intracellular bacteria while minimizing harm to the host cell. A broad diversity of eukaryotes create lysosome- and autophagosome-like vacuoles for recycling cellular material, which can also be used to recover nutrients from killed bacteria (35). Therefore, the production of these toxic vacuoles is likely an ancient antimicrobial strategy for targeting intracellular bacteria.

Eukaryotes can target extracellular bacteria by secreting antibacterial molecules. While this is a common strategy, the molecules themselves can be highly variable in terms of their molecular structures, mode of action, and evolutionary histories. For example, lysozymes, which break down the peptidoglycan cell walls of bacteria, are a highly diverse family of proteins that are broadly distributed, likely present in the LECA (41). Other secreted defenses include diverse antimicrobial peptides (42), iron-scavenging proteins (43,44) and small molecule antibiotics (13,45), which tend to evolve so rapidly that they can be difficult to identify from sequence alone in the absence of functional data. In contrast, some generically toxic molecules, such as ROSs, are produced by many types of eukaryotes and may be released either at the plasma membrane (46,47) or via organelles such as mitochondria or chloroplasts (48,49).

If these intracellular and extracellular antimicrobial strategies are inadequate to control pathogens, eukaryotes can also take drastic measures and undergo programmed cell death (50). Explosive, lytic modes of cell death can release toxic molecules to kill bacteria while alerting neighboring cells to the presence of pathogens (51). Apoptotic cell death can serve to kill intracellular pathogens and trap them within dying host cells. Although programmed cell death might initially seem to be counter-productive to unicellular organisms, apoptotic-like death has been observed across nearly all major eukaryotic lineages, including unicellular taxa (52). Particularly for those species that live in dense populations of cells, programmed cell death may be useful as an altruistic strategy to prevent pathogens from spreading through the population, akin to abortive infection mechanisms that bacteria use to combat bacteriophage (53). All together, the antibacterial immune strategies found in modern-day organisms are an evolutionary

patchwork, with recently-evolved immune mechanisms built upon these widespread, ancient modes of defense.

Finding missing modes of eukaryotic defense

These are some of the cell-intrinsic antibacterial defenses that are the most ancient and broadly distributed across eukaryotic taxa (Table 1), but there is reason to believe that there are many more waiting to be discovered. One reason is that pathogen-interacting genes tend to be some of the fastest evolving genes in host genomes (54,55), due to the strong selective pressures involved in evading pathogen infections (56). This rapid evolution raises challenges for studying the deep ancestry of defense pathways. Many eukaryotic lineages have been studied almost exclusively through sequence data, where probable gene functions are assigned based on conservation to experimentally characterized genes and genomes (largely from animals, plants, or fungi) (57). If cell-intrinsic defense proteins across eukaryotes evolve as rapidly as they do in animals and plants, defense genes are likely to be particularly difficult to identify in diverse, poorly-sampled eukaryotic taxa based on sequence alone. In addition, even within eukaryotic proteins where some conserved domains have been detected (e.g., TIRs and LRRs), additional, as yet uncharacterized, functional domains may lurk in their sequences if these domains are not prevalent in animals, plants, or fungi. In short: beyond the few, extremely conserved strategies (Figure 3), there are almost certainly a lot of defenses that we're missing!

What approaches can be used to discover unknown antibacterial defenses in diverse eukaryotes? An effective strategy would be to expand our experimental approaches to new and emerging eukaryotic model systems. Although the vast majority of microbial eukaryotes are not currently in culture, there are hundreds of cultured species (see Box 2 of (58)), many of which have yet to be probed for their responses to bacteria or bacteria-derived molecules (59). Bioinformatics-based approaches could begin by searching for proteins containing domains known to be associated with immune functions in other organisms, as in the examples of pattern recognition receptors we presented above. Once identified, the expression dynamics of these genes could be probed in response to bacterial exposure. For the expanding list of microbial eukaryotes whose gene expression can be manipulated (60), candidate gene immune functions could be further dissected using overexpression or knockout approaches.

The search for undiscovered antimicrobial functions without homology to known proteins will require a different approach. Because many genes for antimicrobial defense are differentially regulated upon bacterial exposure, genome-wide expression profiling in the presence and absence of bacteria (or bacterial products such as LPS or peptidoglycan) could generate candidate defense genes. Unbiased genetic screens could also be a powerful tool to identify antibacterial genes in those eukaryotes that lack genetic manipulation techniques. For example, for species with small, haploid genomes, random mutagenesis followed by whole-genome sequencing could identify genetic mutations that alter bacterial responses (as in (61)).

These approaches can be useful to understand eukaryotic responses to bacteria, yet eukaryotes must also discriminate among different bacterial species to tailor their antimicrobial responses appropriately. This raises the question: which bacteria should be used to elicit eukaryotic defenses? We propose two possible solutions. First, eukaryotes could be exposed to pathogens with exceptionally broad host ranges such as *Legionella*, which have been used in the lab to infect diverse eukaryotes including Metazoa, Amoebozoa, Ciliophora, and Heterolobosea (62). Alternatively, because eukaryotes are most likely to have adapted to the bacteria they encounter in their local environment, researchers may take advantage of the fact that many microeukaryotes are co-isolated and co-cultured with a community of bacteria. The composition of these microbial communities could be manipulated through nutrient restriction and/or

antibiotic treatments to alter bacterial exposure. Eukaryotes could then be monitored for responses such as the induction of cell death, secretion of antimicrobials, or the production of autophagosome-like membranes. If bacteria in these communities can trigger eukaryotic antibacterial defenses, they could form a natural system to dissect host-pathogen interactions in the lab.

Why is it so important to study this under-sampled diversity of eukaryotic antibacterial defenses? First, we know that bacterial interactions are broadly important to the ecology, physiology, and even the endosymbiotic origin of eukaryotes (1,2,13,15,63), but in most lineages the mechanisms shaping microbial interactions are unknown. Second, diverse eukaryotes can tell us about the origins and evolution of antibacterial defenses. Our eukaryotic ancestors lived with bacteria for hundreds of millions of years before the evolution of animals or plants, and their pre-existing strategies for managing bacterial interactions formed the starting point from which modern-day innate immune systems evolved (4). Learning about this ancestry can help us to make sense of the variety of defensive strategies that we see today. Third, these lineages may serve as rich hunting grounds to discover novel, yet broadly conserved, aspects of cell-intrinsic immunity. Such mechanisms may be easier to discover in organisms without the complications of adaptive immunity or complex interactions among immune cell types. For example, studies of *Dictyostelium* amoebae were critical in uncovering mechanisms of phagocytosis (e.g. (64)). These organisms also encode guanylate-binding proteins (GBPs), a protein family has relatively recently been discovered to function in animal antibacterial defense (65). Because the *Dictyostelium* genome only encodes one GBP as opposed to the dozens in animals, studies in amoebae could avoid some of the complications of functionally characterizing these defenses.

Finally, even if the defense mechanisms we uncover in these eukaryotes are different from what we know in animals and plants, they may still reveal entirely novel biological solutions to the problem of antibacterial immunity. Such mechanisms are bound to inspire new strategies and interventions to combat pathogenic bacteria. They can also broaden our conceptions of how immunity can work, giving us a glimpse into the diverse evolutionary paths that can be followed by defense systems in different eukaryotic lineages.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

We thank Roman Barbalat, Darian Doakes, Mary Fontana, Barbara Kunkel, Jeannette Tenthorey, Russell Vance and Arielle Woznica for their comments on the manuscript. We thank Cédric Berney and Michelle Leger for helpful discussions. We acknowledge an anonymous reviewer for comments that led to improvements on the review. DJR is supported by a postdoctoral fellowship from the Beatriu de Pinós programme of the Government of Catalonia's Secretariat for Universities and Research of the Ministry of Economy and Knowledge. TCL is supported by a Pathway to Independence Award from the National Institute of Allergy and Infectious Disease and the National Institutes of Health (K99 AI139344).

Figures

Figure 1. The spectrum of diversity among eukaryotes. Phylogenetic tree of eukaryotic diversity based on (5) and annotated to show where species descriptions, genome-scale sequencing and studies

of antibacterial defense have been concentrated. Well-known multicellular groups include animals (Metazoa), fungi (within Nucletmycea) and land plants (within Streptophyta). Although plant and animal immune systems have been studied extensively, much of the diversity of eukaryotes remains to be explored; this is especially evident for poorly characterized but extremely diverse lineages such as Dinophyceae and Diplonemea. The placement of the Last Eukaryotic Common Ancestor (LECA) is currently unresolved as indicated by the dashed line; other unresolved branching relationships are drawn as multiple lineages descending from the same most recent ancestor. Branch lengths are not to scale. Estimated numbers of described genera per group for Metazoa are from (66) and all others from (5). Marine OTU counts (operational taxonomic units, roughly comparable to genera/species depending on group) are from *Tara* Oceans, a global sampling of microbial eukaryotic diversity in the surface ocean (6). Counts of publicly available sequence data sets are estimated from the NCBI Genome and Transcriptome Shotgun Assembly (TSA) databases (67). For simplicity, the following groups of eukaryotes with relatively few described species are not shown on the tree: Apusomonadida, Breviatea, Ancyromonadida, CRuMs (the clade including Collodictyonidae, Rigifilida and *Mantamonas*), Ancoracysta, Glaucophyta, Picozoa, Colpodellida, Telonemia, Jakobida, other Discoba, Hemimastigophora, Malawimonadidae. References for studies of antibacterial defense that are not listed in Table 1: Chlorophyta, Cryptista, Haptista, Cercozoa, Chrysophyceae, Pelagophyceae, Euglenida (68–71). “Observation of antibacterial activity” excludes the ability to feed on bacteria as prey.

Figure 2. Conservation and convergence in eukaryotic pattern recognition proteins. A group of protein domains (LRR, TIR, Pkinase, NACHT AND NB-ARC) have been repeatedly reshuffled over the course of eukaryotic evolution. **(a)** Domain architectures (not drawn to scale) of well-studied animal and plant proteins. **(b)** Proteins with similar core domain architectures present outside of animals and plants. For a given domain architecture to be considered present in a lineage, at least two species encoding proteins with the architecture were required; absence is denoted with ‘--’ (lineage names and colors correspond to Figure 1). Protein identifiers beginning with CAMPEP are from MMETSP (8); others are from GenBank. Pfam domains (version 32.0 (72)) are grouped into the following categories based on their primary known functions: binding/pattern recognition (LRR, NRLC4_HD2), nucleotide binding (NACHT, NB-ARC), caspase recruitment (CARD, PYRIN), caspase/peptidase (CHAT), protein-protein interaction (Ank, NOD2_WH, Rx_N, TIR, TPR), kinase (Pkinase; also includes Pkinase_Tyr domains), phosphorylase (PNP_UDP), guanine nucleotide exchange factor (RCC1), phospholipase (PI-PLC-X, PI-PLC-Y). LRR and Ank represent one or more repeated domains. Two of the most frequent fungal NLR-like domain architectures (of more than 30 (72)) are shown. Uncommon domain architectures or those found only in a single species are not shown. Some homologs also contain one or more additional transmembrane domains (not depicted). Plant-type receptor like proteins (RLPs; e.g., tomato Cf-9 (73)) are not shown, as they are thought to act in conjunction with RLKs (34) and are generally composed only of LRRs. Animal-type NLRs do not include the NLR-related protein Apaf-1, which lacks LRR domains (74).

Figure 3. Modes of eukaryotic detection and elimination of bacteria. Cells can kill most internalized bacteria via maturation of the intracellular vacuole into a toxic, nutrient-poor environment. However, bacterial pathogens can evade this fate, either by tolerating these harsh conditions, escaping from the vacuole into the cytosol, or hijacking the vacuole and transforming it into a replicative niche. Hosts possess a number of molecular sensors of bacteria and/or bacteria-derived molecules (such as TLRs, RLKs, NLRs, GBPs, and many others), which can trigger defense signaling cascades. Other sensors can detect abnormal molecules on pathogen-containing vacuoles to trigger vacuole destruction by autophagy. In addition, eukaryotes can kill extracellular bacteria via the secretion of anti-bacterial molecules, the production of reactive oxygen species (ROS) from endosymbiotic organelles, or cell-death-mediated release of toxic molecules.

342
343 **Table**
344
345 **Table 1. Bacterial recognition and defense gene families in eukaryotes**. Gene families or processes
346 known to be involved in defensive response to bacteria that are shared among multiple eukaryotic groups
347 (group names correspond to Figure 1; when names correspond to internal nodes, evidence is present in
348 all groups descending from the node). Genomic evidence is based on searches for diagnostic protein
349 domain architectures in MMETSP data (required to be present in at least 2 species within a lineage, after
350 removal of low levels of inter-species cross-contamination, as in (75)), complemented by literature
351 searches when diagnostic architectures are not available. Hypotheses on the evolution of each domain
352 architecture are constrained by currently available data, and may change as sampling of eukaryotic
353 diversity increases. *: NLR-like proteins have been identified in the genomes of Nucletmycea. **: (76) also
354 found NOS-like proteins in Rhodophyta and Phaeophyceae.
355
356

Process/Gene Family	Known Antibacterial Function(s)	Experimental Evidence in Group(s)	Diagnostic Protein Domains (if any)	Genomic Evidence in Additional Group(s)	Likely Evolved	Non-Immunity Function(s)	Reference(s)
TLRs (Toll-like receptors)	Extracellular pattern recognition	Metazoa	TLR, transmembrane, TIR	Choanoflagellatea	Once, in Choanozoa	Development (Metazoa)	(26,77,78)
Plant-type RLKs (receptor-like kinases, excluding lectins)	Extracellular pattern recognition	Streptophyta	LRR, transmembrane, Pkinase	All, except Apicomplexa	Present in LECA and/or independently evolved in multiple groups	Development, symbiosis (Streptophyta)	(27,79)
NLRs (nucleotide-binding domain, leucine-rich repeat superfamily)	Intracellular pattern recognition	Metazoa, Streptophyta	NACHT or NB-ARC, LRR	Haptista, Dinophyceae, Diatomeae, Nucletmycea*	Independently evolved in multiple groups	Reproduction (Metazoa)	(25,32,80)
STING	Cytosolic cyclic dinucleotide recognition	Metazoa	TMEM173	Choanoflagellatea	Once, in Choanozoa	-	(81)
cGAS	Cytosolic DNA recognition	Metazoa	Mab-21	Choanoflagellatea	Once, in Choanozoa	-	(81)
Lectins	Microbial sugar recognition and agglutination or recognition of host danger signals	Amoebozoa, Metazoa, Streptophyta	Diverse and widely distributed		Present in LECA and/or independently evolved in multiple groups	Adhesion, development, symbiosis	(17,82)
Guanylate-binding proteins (GBPs)	Target vacuolar and cytosolic bacteria for killing	Metazoa	GBP, GBP_C	All, except Rhodophyta, Phaeophyceae, Euglenozoa	Present in LECA	Inhibits cell proliferation, migration	(65,83)

Antimicrobial peptides	Disruption of bacterial membranes and/or cell walls	Amoebozoa, Metazoa, Nucletmycea, Streptophyta, Rhodophyta, Ciliophora, Heterolobosea	Diverse and widely distributed		Present in LECA and/or independently evolved in multiple groups	Gut homeostasis (Metazoa), rhizobial symbiosis (Streptophyta)	(42,84)
Lysozyme	Disruption of bacterial cell walls	Amoebozoa, Metazoa, Nucletmycea, Streptophyta	Diverse and widely distributed		Present in LECA	-	(41)
Nitric oxide synthase (NOS)	Production of NO for bacterial killing	Metazoa	NO_synthase, Flavodoxin, FAD_binding, NAD_binding	Amorphea, Chlorophyta, Haptista, Dinophyceae, Cercozoa, Diatomeae, Chrysophyceae, Pelagophyceae, Euglenida**	Present in LECA	Intracellular and intercellular signaling	(76,85)
NADPH oxidases (NOX, produce reactive oxygen species)	Production of superoxide for bacterial killing	Amoebozoa, Metazoa, Streptophyta, Rhodophyta, Phaeophyceae	transmembrane(s), Ferric_reduct, transmembrane(s), FAD_binding, NAD_binding	All, except Apicomplexa, Metamonada	Present in LECA	Intracellular and intercellular signaling	(46,86–89)
Nramp	Removes metal ions and acidifies vacuoles	Amoebozoa, Metazoa, Streptophyta	Nramp, transmembrane(s)	All, except Metamonada	Present in LECA	Metal ion scavenging for host metabolism	(90)
Mitochondria and chloroplast-mediated defenses	Production of reactive oxygen species	Metazoa, Streptophyta	Not available	-	Present in LECA	Photosynthesis and respiration	(48,49)
Autophagy (incl. xenophagy, etc.)	Sequestering and killing of intracellular bacteria	Amoebozoa, Metazoa, Streptophyta, Kinetoplastea	Numerous genes and pathways	All, but lost in species within Nucletmycea, Rhodophyta, Metamonada	Present in LECA	Disposal of compromised cellular material	(35)

Papers of special interest:

Special interest (*) or outstanding interest (**)

1. Reference 5. Adl SM, Bass D, Lane CE, Lukeš J, Schoch CL, Smirnov A, et al. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. J Eukaryot Microbiol. 2019 Jan 1;66(1):4–119.

* The most up to date taxonomy of eukaryotes, based on a consensus of phylogenetic studies. Includes information on the numbers of described genera in each group and their trophic characteristics.

2. Reference 15. Bjorbækmo MFM, Evenstad A, Røsæg LL, Krabberød AK, Logares R. The planktonic protist interactome: where do we stand after a century of research? bioRxiv. 2019 May 2;587352.
   * A database gathering the results of roughly 500 publications describing roughly 2,500 inter-species interactions of planktonic protists (microbial eukaryotes), including those with bacteria. Categorizes interactions into three types (symbiosis, predation and parasitism).
3. Reference 17. Bellande K, Bono J-J, Savelli B, Jamet E, Canut H. Plant Lectins and Lectin Receptor-Like Kinases: How Do They Sense the Outside? Int J Mol Sci. 2017;18(6).
   * A comprehensive review of lectins and lectin receptor-like kinases, summarizing the evolution of different classes of lectins and their domain architectures.
4. Reference 18. Dinh C, Farinholt T, Hirose S, Zhuchenko O, Kuspa A. Lectins modulate the microbiota of social amoebae. Science. 2018 Jul 27;361(6400):402–6.
   ** This paper shows that an amoeba secreted lectin alters grazing and killing of bacteria. It also allows for amoeba cells to harbor intracellular live bacteria following phagocytosis. Strikingly, this bacterial carriage leads to high rates of amoeba transformation, as plasmids from the bacteria are transferred to and expressed within the amoebae.
5. Reference 23. Gao Y, Wang W, Zhang T, Gong Z, Zhao H, Han G-Z. Out of Water: The Origin and Early Diversification of Plant R-Genes. Plant Physiol. 2018 Mar 21;pp.00185.2018.
   ** A thorough survey of the diversification of *R*-gene (resistance gene) proteins and domain architectures in Archaeplastida, including plant-type NLRs in numerous early-branching lineages.
6. Reference 25. Urbach JM, Ausubel FM. The NBS-LRR architectures of plant R-proteins and metazoan NLRs evolved in independent events. Proc Natl Acad Sci. 2017 Jan 31;114(5):1063–8.
   ** A detailed study of the evolutionary history of plant and animal NLRs (nucleotide-binding domain, leucine-rich repeat superfamily), demonstrating that their domain architectures evolved at least twice independently.
7. Reference 26. Richter DJ, Fozouni P, Eisen MB, King N. Gene family innovation, conservation and loss on the animal stem lineage. Telford MJ, editor. eLife. 2018 May 31;7:e34226.
   * Survey of transcriptomes across Choanoflagellatea, documenting a history of global gene gain and loss across Choanozoa. Includes discovery of kinase TLRs and assessment of TLR pathway conservation in animals and choanoflagellates.
8. Reference 54. Enard D, Cai L, Gwennap C, Petrov DA. Viruses are a dominant driver of protein adaptation in mammals. McVean G, editor. eLife. 2016 May 17;5:e12469.
   * See reference 55, Shultz and Sackton (2019).

   Reference 55. Shultz AJ, Sackton TB. Immune genes are hotspots of shared positive selection across birds and mammals. Landry CR, Tautz D, editors. eLife. 2019 Jan 8;8:e41815.
   ** Together with Enard et al (2016), this paper performed a genome-wide screen for genes under positive selection, finding that the strongest adaptation is in pathogen-interacting genes. The same gene families in birds and mammals are independently experiencing these selective pressures.
9. Reference 65. Dunn JD, Bosmani C, Barisch C, Raykov L, Lefrançois LH, Cardenal-Muñoz E, et al. Eat Prey, Live: Dictyostelium discoideum As a Model for Cell-Autonomous Defenses. Front Immunol. 2018;8:1906.
   ** Comprehensive review comparing and contrasting modes of bacterial antagonism present in mammalian phagocytes and *Dictyostelium* amoebae.

10. Reference 70. Cepas V, López Y, Gabasa Y, Martins CB, Ferreira JD, Correia MJ, et al. Inhibition of Bacterial and Fungal Biofilm Formation by 675 Extracts from Microalgae and Cyanobacteria. Antibiotics. 2019 Jun 12;8(2):77.

    * Detected antibacterial activity within extracts from cell cultures of over 200 diverse microalgal species representing more than 10 different eukaryotic groups (including Cercozoa, Chlorophyta, Cryptista, Euglenida, Haptista, Myzozoa, Ochrophyta and Rhodophyta).

11. Reference 77. Brennan JJ, Messerschmidt JL, Williams LM, Matthews BJ, Reynoso M, Gilmore TD. Sea anemone model has a single Toll-like receptor that can function in pathogen detection, NF-κB signal transduction, and development. Proc Natl Acad Sci. 2017 Nov 21;114(47):E10122–31.

    * Among the first studies to characterize Toll-like receptor function in an early-branching animal lineage, the cnidarian *Nematostella vectensis*, finding that key interactions with intracellular binding partners are conserved with bilaterian animals and that the TLR also functions in early embryonic development.

References

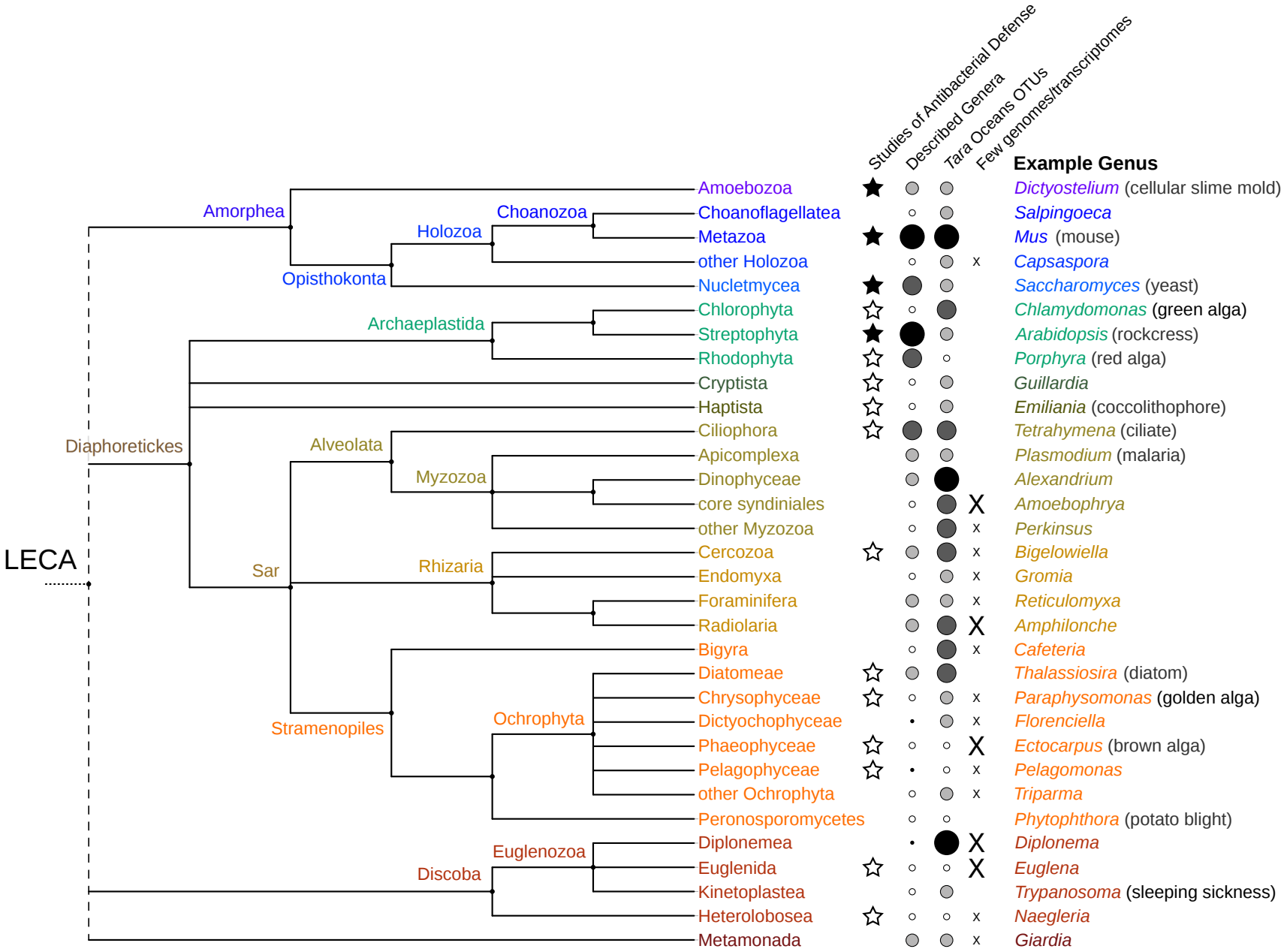
1. Knoll AH. Life on a Young Planet: the First Three Billion Years of Evolution on Earth. 2015. 296 p.
2. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: The unseen majority. Proc Natl Acad Sci. 1998 Jun 9;95(12):6578–83.
3. Medzhitov R, Janeway CA. Innate Immunity: The Virtues of a Nonclonal System of Recognition. Cell. 1997 Oct;91(3):295–8.
4. Danilova N. The evolution of immune mechanisms. J Exp Zoolog B Mol Dev Evol. 2006 Nov 15;306B(6):496–520.
5. Adl SM, Bass D, Lane CE, Lukeš J, Schoch CL, Smirnov A, et al. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. J Eukaryot Microbiol. 2019 Jan 1;66(1):4–119.
6. de Vargas C, Audic S, Henry N, Decelle J, Mahe F, Logares R, et al. Eukaryotic plankton diversity in the sunlit ocean. Science. 2015 May 22;348(6237):1261605–1261605.
7. Mahé F, de Vargas C, Bass D, Czech L, Stamatakis A, Lara E, et al. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. Nat Ecol Evol. 2017 Mar 20;1:0091.
8. Keeling PJ, Burki F, Wilcox HM, Allam B, Allen EE, Amaral-Zettler LA, et al. The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the Functional Diversity of Eukaryotic Life in the Oceans through Transcriptome Sequencing. Roberts RG, editor. PLoS Biol. 2014 Jun 24;12(6):e1001889.
9. Monroe KM, McWhirter SM, Vance RE. Identification of Host Cytosolic Sensors and Bacterial Factors Regulating the Type I Interferon Response to Legionella pneumophila. Isberg RR, editor. PLoS Pathog. 2009 Nov 20;5(11):e1000665.
10. tenOever BR. The Evolution of Antiviral Defense Systems. Cell Host Microbe. 2016 Feb;19(2):142–9.
11. Zhao J-H, Guo H-S. Trans-kingdom RNA interactions drive the evolutionary arms race between hosts and pathogens. Curr Opin Genet Dev. 2019 Oct;58–59:62–9.
12. Shiner EK, Rumbaugh KP, Williams SC. Interkingdom signaling: Deciphering the language of acyl homoserine lactones. FEMS Microbiol Rev. 2005 Nov 1;29(5):935–47.
13. Amin SA, Parker MS, Armbrust EV. Interactions between Diatoms and Bacteria. Microbiol Mol Biol Rev. 2012 Sep 1;76(3):667–84.
14. Haldar K, Kamoun S, Hiller NL, Bhattacharje S, van Ooij C. Common infection strategies of

463 pathogenic eukaryotes. Nat Rev Microbiol. 2006 Dec;4(12):922–31.
464 15. Bjorbækmo MFM, Evenstad A, Røsæg LL, Krabberød AK, Logares R. The planktonic protist
465 interactome: where do we stand after a century of research? bioRxiv. 2019 May 2;587352.
466 16. Kilpatrick D. Animal lectins: a historical introduction and overview. Biochim Biophys Acta
467 BBA - Gen Subj. 2002 Sep 19;1572(2–3):187–97.
468 17. Bellande K, Bono J-J, Savelli B, Jamet E, Canut H. Plant Lectins and Lectin Receptor-Like
469 Kinases: How Do They Sense the Outside? Int J Mol Sci. 2017;18(6).
470 18. Dinh C, Farinholt T, Hirose S, Zhuchenko O, Kuspa A. Lectins modulate the microbiota of
471 social amoebae. Science. 2018 Jul 27;361(6400):402–6.
472 19. Margolis SR, Wilson SC, Vance RE. Evolutionary Origins of cGAS-STING Signaling. Trends
473 Immunol. 2017 Oct;38(10):733–43.
474 20. Whiteley AT, Eaglesham JB, de Oliveira Mann CC, Morehouse BR, Lowey B, Nieminen EA,
475 et al. Bacterial cGAS-like enzymes synthesize diverse nucleotide signals. Nature. 2019
476 Mar;567(7747):194–9.
477 21. Ausubel FM. Are innate immune signaling pathways in plants and animals conserved? Nat
478 Immunol. 2005 Oct;6(10):973–9.
479 22. Jacob F, Vernaldi S, Maekawa T. Evolution and Conservation of Plant NLR Functions. Front
480 Immunol. 2013;4:297.
481 23. Gao Y, Wang W, Zhang T, Gong Z, Zhao H, Han G-Z. Out of Water: The Origin and Early
482 Diversification of Plant R-Genes. Plant Physiol. 2018 Mar 21;pp.00185.2018.
483 24. Segonzac C, Zipfel C. Activation of plant pattern-recognition receptors by bacteria. Curr
484 Opin Microbiol. 2011 Feb;14(1):54–61.
485 25. Urbach JM, Ausubel FM. The NBS-LRR architectures of plant R-proteins and metazoan
486 NLRs evolved in independent events. Proc Natl Acad Sci. 2017 Jan 31;114(5):1063–8.
487 26. Richter DJ, Fozouni P, Eisen MB, King N. Gene family innovation, conservation and loss on
488 the animal stem lineage. Telford MJ, editor. eLife. 2018 May 31;7:e34226.
489 27. Diévart A, Gilbert N, Droc G, Attard A, Gourgues M, Guiderdoni E, et al. Leucine-Rich
490 repeat receptor kinases are sporadically distributed in eukaryotic genomes. BMC Evol Biol.
491 2011;11(1):367.
492 28. Jones JDG, Vance RE, Dangl JL. Intracellular innate immune surveillance devices in plants
493 and animals. Science. 2016 Dec 2;354(6316):aaf6395–aaf6395.
494 29. Janeway CA, Medzhitov R. Innate Immune Recognition. Annu Rev Immunol. 2002
495 Apr;20(1):197–216.
496 30. Shalini S, Dorstyn L, Dawar S, Kumar S. Old, new and emerging functions of caspases. Cell
497 Death Differ. 2015 Apr;22(4):526–39.
498 31. Walk A, Callahan J, Srisawangvong P, Leuschner J, Samaroo D, Cassilly D, et al.
499 Lipopolysaccharide enhances bactericidal activity in Dictyostelium discoideum cells. Dev
500 Comp Immunol. 2011 Aug;35(8):850–6.
501 32. Uehling J, Deveau A, Paoletti M. Do fungi have an innate immune response? An NLR-
502 based comparison to plant and animal immune systems. Zipfel C, editor. PLOS Pathog.
503 2017 Oct 26;13(10):e1006578.
504 33. Kombrink A, Tayyrov A, Essig A, Stöckli M, Micheller S, Hintze J, et al. Induction of
505 antibacterial proteins and peptides in the coprophilous mushroom Coprinopsis cinerea in
506 response to bacteria. ISME J. 2019 Mar;13(3):588–602.
507 34. Zipfel C. Plant pattern-recognition receptors. Trends Immunol. 2014 Jul;35(7):345–51.
508 35. Duszenko M, Ginger ML, Brennand A, Gualdrón-López M, Colombo MI, Coombs GH, et al.
509 Autophagy in protists. Autophagy. 2011 Oct 27;7(2):127–58.
510 36. de Duve C. The Lysosome Concept. In: de Reuck AVS, Cameron MP, editors. Novartis
511 Foundation Symposia [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 1963. p. 1–35.
512 Available from: https://dx.doi.org/10.1002/9780470715314.ch1
513 37. Gatica D, Lahiri V, Klionsky DJ. Cargo recognition and degradation by selective autophagy.

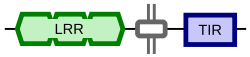
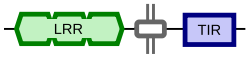
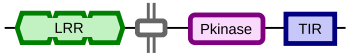
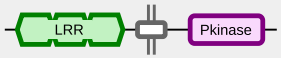
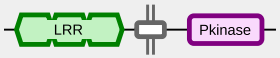
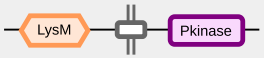
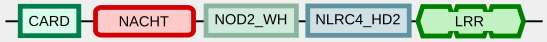
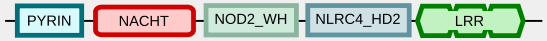
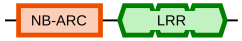
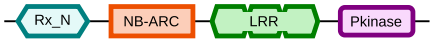
Nat Cell Biol. 2018 Mar;20(3):233–42.
38. Cardenal-Muñoz E, Arafah S, López-Jiménez AT, Kicka S, Falaise A, Bach F, et al. Mycobacterium marinum antagonistically induces an autophagic response while repressing the autophagic flux in a TORC1- and ESX-1-dependent manner. Lewinsohn DM, editor. PLoS Pathog. 2017 May 17;13(4):e1006344.
39. Pizarro-Cerdá J, Cossart P. Bacterial Adhesion and Entry into Host Cells. Cell. 2006 Feb 24;124(4):715–27.
40. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunol Rev. 2015 Mar;264(1):182–203.
41. Monzingo AF, Marcotte EM, Hart PJ, Robertas JD. Chitinases, chitosanases, and lysozymes can be divided into procaryotic and eucaryotic families sharing a conserved core. Nat Struct Biol. 1996 Feb;3(2):133–40.
42. Wang G, Li X, Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Res. 2016 Feb 3;44(D1):D1087–93.
43. Núñez G, Sakamoto K, Soares MP. Innate Nutritional Immunity. J Immunol. 2018 Jul 1;201(1):11–8.
44. Verbon EH, Trapet PL, Stringlis IA, Kruijs S, Bakker PAHM, Pieterse CMJ. Iron and Immunity. Annu Rev Phytopathol. 2017 Aug 4;55(1):355–75.
45. Kubohara Y, Shiratsuchi Y, Ishigaki H, Takahashi K, Oshima Y, Kikuchi H. Antimicrobial Activities of Dictyostelium Differentiation-Inducing Factors and Their Derivatives. Biomolecules. 2019 Jun;9(5):163.
46. Qi J, Wang J, Gong Z, Zhou J-M. Apoplastic ROS signaling in plant immunity. Curr Opin Plant Biol. 2017 Aug;38:92–100.
47. Nguyen GT, Green ER, Mecsas J. Neutrophils to the ROScue: Mechanisms of NADPH Oxidase Activation and Bacterial Resistance. Front Cell Infect Microbiol. 2017 Aug 25;7:373.
48. Serrano I, Audran C, Rivas S. Chloroplasts at work during plant innate immunity. J Exp Bot. 2016 Jun;67(13):3845–54.
49. Sander LE, Garaude J. The mitochondrial respiratory chain: A metabolic rheostat of innate immune cell-mediated antibacterial responses. Mitochondrion. 2018 Jul;41:28–36.
50. Jorgensen I, Rayamajhi M, Miao EA. Programmed cell death as a defence against infection. Nat Rev Immunol. 2017 Mar;17(3):151–64.
51. Liu X, Lieberman J. Chapter Three - A Mechanistic Understanding of Pyroptosis: The Fiery Death Triggered by Invasive Infection. In: Alt FW, editor. Advances in Immunology [Internet]. Academic Press; 2017. p. 81–117. Available from: http://www.sciencedirect.com/science/article/pii/S0065277617300184
52. Deponte M. Programmed cell death in protists. Biochim Biophys Acta BBA - Mol Cell Res. 2008 Jul;1783(7):1396–405.
53. Shub DA. Bacterial Viruses: Bacterial altruism? Curr Biol. 1994 Jun 1;4(6):555–6.
54. Enard D, Cai L, Gwennap C, Petrov DA. Viruses are a dominant driver of protein adaptation in mammals. McVean G, editor. eLife. 2016 May 17;5:e12469.
55. Shultz AJ, Sackton TB. Immune genes are hotspots of shared positive selection across birds and mammals. Landry CR, Tautz D, editors. eLife. 2019 Jan 8;8:e41815.
56. Daugherty MD, Malik HS. Rules of Engagement: Molecular Insights from Host-Virus Arms Races. Annu Rev Genet. 2012;46(1):677–700.
57. Radivojac P, Clark WT, Oron TR, Schnoes AM, Wittkop T, Sokolov A, et al. A large-scale evaluation of computational protein function prediction. Nat Methods. 2013 Mar;10(3):221–7.
58. del Campo J, Sieracki ME, Molestina R, Keeling P, Massana R, Ruiz-Trillo I. The others: our biased perspective of eukaryotic genomes. Trends Ecol Evol. 2014 May 1;29(5):252–9.
59. Abida H, Ruchaud S, Rios L, Humeau A, Probert I, De Vargas C, et al. Bioprospecting Marine Plankton. Mar Drugs. 2013 Nov 14;11(11):4594–611.

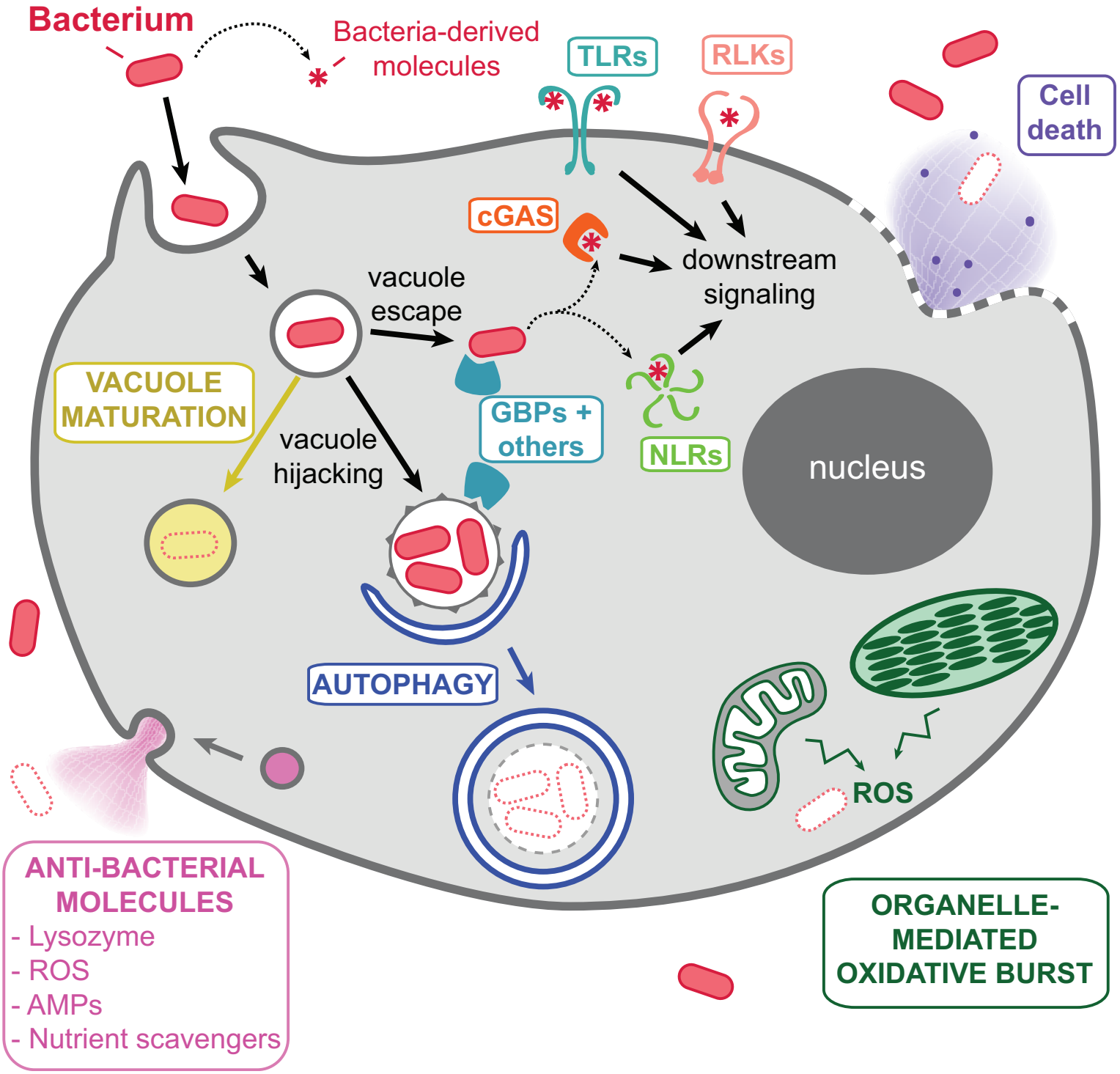
60. Waller RF, Cleves PA, Rubio-Brotons M, Woods A, Bender SJ, Edgcomb V, et al. Strength in numbers: Collaborative science for new experimental model systems. PLOS Biol. 2018 Jul 2;16(7):e2006333.
61. Levin TC, Greaney AJ, Wetzel L, King N. The rosetteless gene controls development in the choanoflagellate S. rosetta. Sánchez Alvarado A, editor. eLife. 2014 Oct 9;3:e04070.
62. Boamah DK, Zhou G, Ensminger AW, O'Connor TJ. From Many Hosts, One Accidental Pathogen: The Diverse Protozoan Hosts of Legionella. Front Cell Infect Microbiol [Internet]. 2017 [cited 2019 Aug 28];7. Available from: https://www.frontiersin.org/articles/10.3389/fcimb.2017.00477/full
63. Ramanan R, Kim B-H, Cho D-H, Oh H-M, Kim H-S. Algae–bacteria interactions: Evolution, ecology and emerging applications. Biotechnol Adv. 2016 Feb 2;34(1):14–29.
64. Seastone DJ, Zhang L, Buczynski G, Rebstein P, Weeks G, Spiegelman G, et al. The Small Mr Ras-like GTPase Rap1 and the Phospholipase C Pathway Act to Regulate Phagocytosis in Dictyostelium discoideum. Pfeffer SR, editor. Mol Biol Cell. 1999 Feb;10(2):393–406.
65. Dunn JD, Bosmani C, Barisch C, Raykov L, Lefrançois LH, Cardenal-Muñoz E, et al. Eat Prey, Live: Dictyostelium discoideum As a Model for Cell-Autonomous Defenses. Front Immunol. 2018;8:1906.
66. Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B. How Many Species Are There on Earth and in the Ocean? Mace GM, editor. PLoS Biol. 2011 Aug 23;9(8):e1001127.
67. NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2016 Jan 4;44(D1):D7–19.
68. Falaise C, François C, Travers M-A, Morga B, Haure J, Tremblay R, et al. Antimicrobial Compounds from Eukaryotic Microalgae against Human Pathogens and Diseases in Aquaculture. Mar Drugs. 2016 Sep 2;14(9):159.
69. Shannon E, Abu-Ghannam N. Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. Mar Drugs. 2016 Apr 22;14(4):81.
70. Cepas V, López Y, Gabasa Y, Martins CB, Ferreira JD, Correia MJ, et al. Inhibition of Bacterial and Fungal Biofilm Formation by 675 Extracts from Microalgae and Cyanobacteria. Antibiotics. 2019 Jun 12;8(2):77.
71. Davison JR, Bewley CA. Antimicrobial Chrysophaentin Analogs Identified from Laboratory Cultures of the Marine Microalga *Chrysophaeum taylorii*. J Nat Prod. 2019 Jan 25;82(1):148–53.
72. Dyrka W, Lamacchia M, Durrens P, Kobe B, Daskalov A, Paoletti M, et al. Diversity and Variability of NOD-Like Receptors in Fungi. Genome Biol Evol. 2014 Dec;6(12):3137–58.
73. Jones D, Thomas C, Hammond-Kosack K, Balint-Kurti P, Jones J. Isolation of the tomato Cf-9 gene for resistance to Cladosporium fulvum by transposon tagging. Science. 1994 Nov 4;266(5186):789–93.
74. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a Human Protein Homologous to C. elegans CED-4, Participates in Cytochrome c–Dependent Activation of Caspase-3. Cell. 1997 Aug;90(3):405–13.
75. Marron AO, Ratcliffe S, Wheeler GL, Goldstein RE, King N, Not F, et al. The Evolution of Silicon Transport in Eukaryotes. Mol Biol Evol. 2016 Dec;33(12):3226–48.
76. Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, et al. Occurrence, structure, and evolution of nitric oxide synthase–like proteins in the plant kingdom. Sci Signal. 2016 Mar 1;9(417):re2–re2.
77. Brennan JJ, Messerschmidt JL, Williams LM, Matthews BJ, Reynoso M, Gilmore TD. Sea anemone model has a single Toll-like receptor that can function in pathogen detection, NF-κB signal transduction, and development. Proc Natl Acad Sci. 2017 Nov 21;114(47):E10122–31.
78. Brandt JP, Ringstad N. Toll-like Receptor Signaling Promotes Development and Function of Sensory Neurons Required for a C. elegans Pathogen-Avoidance Behavior. Curr Biol. 2015

616 Aug;25(17):2228–37.
617 79. Antolín-Llovera M, Ried MK, Binder A, Parniske M. Receptor Kinase Signaling Pathways in
618 Plant-Microbe Interactions. Annu Rev Phytopathol. 2012 Sep 8;50(1):451–73.
619 80. Kufer TA, Sansonetti PJ. NLR functions beyond pathogen recognition. Nat Immunol. 2011
620 Feb;12(2):121–8.
621 81. Wu X, Wu F-H, Wang X, Wang L, Siedow JN, Zhang W, et al. Molecular evolutionary and
622 structural analysis of the cytosolic DNA sensor cGAS and STING. Nucleic Acids Res. 2014
623 Jul 29;42(13):8243–57.
624 82. Sharon N. Lectins. In: John Wiley & Sons, Ltd, editor. eLS [Internet]. Chichester, UK: John
625 Wiley & Sons, Ltd; 2009 [cited 2019 Jun 27]. Available from:
626 http://doi.wiley.com/10.1002/9780470015902.a0000708.pub2
627 83. Britzen-Laurent N, Herrmann C, Naschberger E, Croner RS, Stürzl M. Pathophysiological
628 role of guanylate-binding proteins in gastrointestinal diseases. World J Gastroenterol.
629 2016;22(28):6434.
630 84. Maróti G, Kereszt A, Kondorosi É, Mergaert P. Natural roles of antimicrobial peptides in
631 microbes, plants and animals. Res Microbiol. 2011 May;162(4):363–74.
632 85. Andreakis N, D'Aniello S, Albalat R, Patti FP, Garcia-Fernandez J, Procaccini G, et al.
633 Evolution of the Nitric Oxide Synthase Family in Metazoans. Mol Biol Evol. 2011 Jan
634 1;28(1):163–79.
635 86. Kawahara B, Quinn MT, Lambeth JD. Molecular evolution of the reactive oxygen-generating
636 NADPH oxidase (Nox/Duox) family of enzymes. BMC Evol Biol. 2007;7(1):109.
637 87. Egan S, Fernandes ND, Kumar V, Gardiner M, Thomas T. Bacterial pathogens, virulence
638 mechanism and host defence in marine macroalgae: Bacterial pathogens of macroalgae.
639 Environ Microbiol. 2014 Apr;16(4):925–38.
640 88. Geiszt M, Leto TL. The Nox Family of NAD(P)H Oxidases: Host Defense and Beyond. J Biol
641 Chem. 2004 Dec 10;279(50):51715–8.
642 89. Wang Y-J, Wei X-Y, Jing X-Q, Chang Y-L, Hu C-H, Wang X, et al. The Fundamental Role of
643 NOX Family Proteins in Plant Immunity and Their Regulation. Int J Mol Sci. 2016
644 Jul;17(6):805.
645 90. Nevo Y, Nelson N. The NRAMP family of metal-ion transporters. Biochim Biophys Acta BBA
646 - Mol Cell Res. 2006 Jul;1763(7):609–20.


Studies of Antibacterial Defense
Described Genera
Tara Oceans OTUs
Few genomes/transcriptomes
Example Genus
LECA
Diaphoretickes
Amorphea
Opisthokonta
Holozoa
Choanozoa
Archaeplastida
Sar
Alveolata
Myzozoa
Rhizaria
Stramenopiles
Ochrophyta
Euglenozoa
Discoba
Amoebozoa
Choanoflagellatea
Metazoa
other Holozoa
Nucletmycea
Chlorophyta
Streptophyta
Rhodophyta
Cryptista
Haptista
Ciliophora
Apicomplexa
Dinophyceae
core syndiniales
other Myzozoa
Cercozoa
Endomyxa
Foraminifera
Radiolaria
Bigyra
Diatomeae
Chrysophyceae
Dictyochophyceae
Phaeophyceae
Pelagophyceae
other Ochrophyta
Peronosporomycetes
Diplonemea
Euglenida
Kinetoplastea
Heterolobosea
Metamonada
Dictyostelium (cellular slime mold)
Salpingoeca
Mus (mouse)
Capsaspora
Saccharomyces (yeast)
Chlamydomonas (green alga)
Arabidopsis (rockcress)
Porphyra (red alga)
Guillardia
Emiliania (coccolithophore)
Tetrahymena (ciliate)
Plasmodium (malaria)
Alexandrium
Amoebophrya
Perkinsus
Bigelowiella
Gromia
Reticulomyxa
Amphilonche
Cafeteria
Thalassiosira (diatom)
Paraphysomonas (golden alga)
Florenciella
Ectocarpus (brown alga)
Pelagomonas
Triparma
Phytophthora (potato blight)
Diplonema
Euglena
Trypanosoma (sleeping sickness)
Naegleria
Giardia
Mechanisms of antibacterial defense studied
Observation of antibacterial activity
<10 taxa
10-100 taxa
101-1,000 taxa
1,001-10,000 taxa
>10,000 taxa
1-5 genomes/ transcriptomes
≤ 15 genomes/ transcriptomes

a **b**

Domain architecture in animals or plants	Example protein, Genus	Domain architecture in other eukaryotes	Example protein, Genus	Lineage(s) present outside animals/plants
Animal TLRs: TIR, transmembrane, LRR				
	NP_991388 (TLR11), *Mus*		XP_004992000, *Salpingoeca*	Choanoflagellatea
--	--		CAMPEP_0206309248, *Acanthoeca*	Choanoflagellatea
Plant receptor-like kinases: LRR/LysM, transmembrane, Pkinase				
	NP_199445 (FLS2), *Arabidopsis*		CAMPEP_0179057052, *Pyrodinium*	All, except Apicomplexa
	NP_566689 (CERK1), *Arabidopsis*	--	--	
Amoebozoan TIR: RCC1, Ank, TIR				
--	--		XP_636358 (tirA), *Dictyostelium*	Amoebozoa
Animal NLRs: NACHT and LRR				
	NP_001157214 (NLRX1), *Mus*		CAMPEP_0169199868, *Karlodinium*	Choanoflagellatea, Haptista Dinophyceae, Diatomeae
	XP_766317 (NOD1), *Mus*	--	--	
	NP_766484 (NLRP4a), *Mus*	--	--	
--	--		CAMPEP_0182949504, *Acanthoeca*	Choanoflagellatea
--	--		CAMPEP_0116146574, *Pseudo-nitzschia*	Diatomeae
Plant NLRs: NB-ARC and LRR				
	NP_001321385 (RFL1), *Arabidopsis*		CAMPEP_0172974580, *Phaeocystis*	Haptista
	NP_199338 (RPS4), *Arabidopsis*	--	--	
	XP_003576423, *Brachypodium*	--	--	
	XP_024379207, *Physcomitrella*	--	--	
--	--		CAMPEP_0183108492, *Prymnesium*	Haptista
Fungal NLRs: central NACHT or NB-ARC				
--	--		XP_011325800, *Fusarium*	Nucletmycea
--	--		XP_001391272, *Aspergillus*	Nucletmycea


Bacterium
Bacteria-derived molecules
TLRs
RLKs
Cell death
cGAS
downstream signaling
vacuole escape
VACUOLE MATURATION
vacuole hijacking
GBPs + others
NLRs
nucleus
AUTOPHAGY
ROS
ANTI-BACTERIAL MOLECULES
- Lysozyme
- ROS
- AMPs
- Nutrient scavengers
ORGANELLE-MEDIATED OXIDATIVE BURST