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Plant and Animal Sensors of Conserved Microbial Signatures

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The last common ancestor of plants and animals may have lived 1 billion years ago. Plants and animals have occasionally exchanged genes but, for the most part, have countered selective pressures independently. Microbes (bacteria, eukaryotes, and viruses) were omnipresent threats, influencing the direction of multicellular evolution. Receptors that detect molecular signatures of infectious organisms mediate awareness of nonself and are integral to host defense in plants and animals alike. The discoveries leading to elucidation of these receptors and their ligands followed a similar logical and methodological pathway in both plant and animal research.

The mechanisms of plant and animal defense against microbes have historically been presumed to be separate and distinct (fig. S1). Beginning around the 1870s, studies of animal responses to infection revealed the existence of both "natural" or innate immunity, which involved the cells and molecules mediating host inflammatory responses, and adaptive immunity, which permitted the generation of cellular receptors with immense diversity and exquisite specificity for foreign macromolecules of almost any kind. Lacking phagocytes, lymphocytes, antibodies, and many other parts of the animal armamentarium, it seemed that the plant response to disease must use a fundamentally different strategy.

However, discoveries over the past 15 years demonstrate that the mechanisms that allow plants and animals to resist infection show impressive structural and strategic similarity (Fig. 1). Remarkably, the elucidation of these mechanisms followed a common approach involving a concerted attack on the same basic questions: What molecules are recognized by the host as signatures of infection? What receptors mediate recognition? These questions were ultimately answered by classical genetic studies.

Host Receptors that Recognize Microbial Signature Molecules

In biological systems, recognition implies the existence of one or more specific receptors that sense a molecular change in the environment and transduce this change at the cellular level, eliciting a response.

Plant host defense. Plant biology led the way in the discovery of proteins that directly sense infection. As in studies of interactions between lipopolysaccharide (LPS) and Toll-like receptor 4 (TLR4) (described below), genetic data preceded and accurately predicted physical evidence of receptor:ligand interaction. In 1946, Flor, working with the rust disease of flax, proposed the "gene-for-gene hypothesis" based on genetic analyses of variation within host and pathogen populations: When corresponding pathogen avirulence (*avr*) and host resistance (*R*) genes are present in each organism, recognition occurs and defense responses are activated, limiting infection (*1*). Flor's model presumed that specific sensors for microbial molecules, termed elicitors, or avr gene products, were present in immunocompetent hosts.

An intense hunt began in the 1980s to identify the genes encoding these receptors and their corresponding elicitors. Biochemists used innovative cell culture bioassays to monitor early responses of plant cells to diverse microbial molecules (*2*). They identified specific binding sites for elicitors on intact plant cells and on isolated plasma membranes, suggesting the presence of specific host receptors. However, as in mammalian systems, attempts to purify these receptors were unsuccessful.

In the 1990s, an avalanche of genetic experiments led to the isolation of the first *R* genes from multiple plant species. These discoveries established that diverse molecules and mechanisms govern the resistance phenotypes described by Flor and that the resistance response was more complex than previously realized. Some scientists predicted that certain *R* gene products might in fact be equivalent to the receptors that the biochemists were seeking (*2*). However, many plant biologists saw elicitor perception as a field of its own, little overlapping with the field of gene-for-gene resistance. One reason for this philosophical divergence was that many *R* genes conferred resistance to specific races of pathogens carrying *avr* genes thought to be highly variable, whereas elicitors were thought to be more broadly conserved. Isolation of diverse classes of *R* genes allowed direct testing of these disparate views.

Many *R* genes were shown to encode NLRs [nucleotide-binding domain, leucine-rich repeat (LRR)–containing intracellular proteins]. Others encoded kinase domains or receptor-like proteins, lacking kinases. Some R proteins were later shown to directly or indirectly perceive highly variable *avr* gene products, which are secreted directly into the plant cell through bacterial type III secretion systems (TTSS). One *R* gene, *Xa21* (*Xanthomonas* resistance 21), is of special interest in the context of this review, not only because it was predicted to recognize a conserved microbial determinant common to most if not all *X. oryzae* pv. *oryzae* (*Xoo*) strains, a property not previously noted in studies of most other *R* genes, but also because of its distinctive structure: a receptor kinase with LRRs in the extracellular domain (*3*). The cytoplasmic domain of XA21 belongs to the non–arginine-aspartate (non-RD) subclass of kinases. In contrast to RD kinases that carry a conserved arginine immediately preceding the catalytic aspartate, non-RD kinases typically carry a cysteine or glycine in place of the arginine. The non-RD domain was later shown to be a hallmark of kinases associated with early signaling events in both plant and animal innate immunity (*4*). LRR kinases that function in development fall into the RD class.

The predicted structure of XA21 immediately suggested a mechanism of action, in which the extracellular domain would engage a microbial elicitor leading to signal transduction by the cytoplasmic domain (Fig. 1). However, the structure of XA21 gave no clue as to nature of the microbially derived molecule, which was not isolated for another 14 years. We now know that XA21 binds to a highly conserved type I secreted peptide called $AxY^{S}22$ and that sulfation is critical for recognition (*5*).

The availability of the finished *Arabidopsis* and rice genome sequences (representative species of the two major classes of flowering plants, monocots and dicots) revealed that *Xa21* represented a large class of predicted host sensors with non-RD kinase domains. These include 35 proteins encoded in the *Arabidopsis* genome and 328 proteins encoded in the rice genome (*4*). Among these are the *Arabidopsis* proteins FLS2 and EFR (Ef-Tu receptor) and the rice proteins XA26, Pid2, and XA21 (Fig. 1).

The discovery of FLS2 in 2000, also by positional cloning and transgenic complementation of a null genetic background, was of particular importance to plant biologists because it was the first demonstration that a plant host sensor could directly bind a conserved microbial signature [see Supporting Online Material (SOM)]. Boller and colleagues showed that bacterial flagellin, or derivatives of the conserved flg22 epitope present in its N-terminal region, could elicit defense responses in *Arabidopsis* seedlings carrying the FLS2 receptor. With the discovery in 2001 that TLR5 served as the animal receptor

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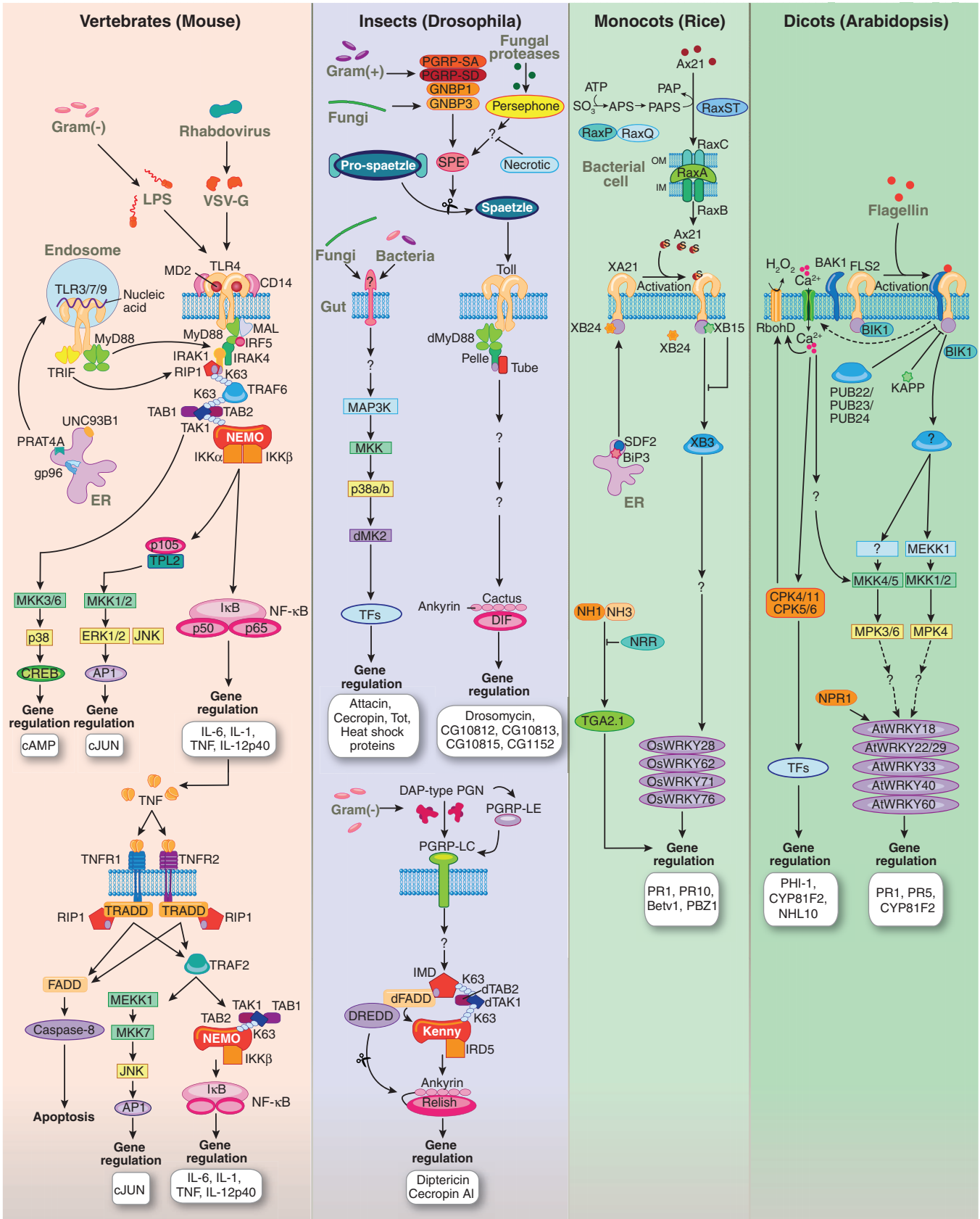

Fig. 1. Host-sensor mediated immune pathways in plants and animals. The pathways depicted are for mouse, fruit fly, rice, and *Arabidopsis*. (See SOM for details.)

for flagellin (*6*), a clear, irrefutable picture emerged: Plants and animals use similar types of cell surface sensors to detect conserved microbial signatures. These host sensors are often called pattern recognition receptors (PRRs) because they recognize conserved microbial-associated molecular patterns (MAMPs).

With the demonstration that XA21 binds a highly conserved *Xoo*-derived peptide (*5*) and the discovery that a mutation in FLS2 rendered *Arabidopsis* susceptible to the bacterial pathogen *Pseudomonas syringae* (*7*), the plant biology community began to accept that some classically defined resistance genes such as *Xa21* encode receptors for microbial signatures and that FLS2 functions in host resistance (*8*). In addition to the well characterized XA21, FLS2, and EFR host sensors, several receptor-like proteins and receptor kinases have been shown or hypothesized to be involved in recognition of conserved microbial signatures (see SOM). Conversely, a number of conserved microbial signature molecules, including proteins, fatty acids, and oligosaccharides, have been identified from bacteria and oomycetes, but their receptors have not yet been identified.

Insect host defense. The last common ancestor of insects and mammals is believed to have lived 640 million years ago (Ma), not long after the divergence of plants and animals. Work in *Drosophila* established that Toll, originally known for its function in development and its ability to elicit a nuclear factor κB (NF-κB) response, is a key transducer of responses to fungal and Gram-positive bacterial infection (*9*). Like XA21, FLS2, and EFR, Toll carries LRRs in the predicted extracellular domain and signals through a non-RD kinase called Pelle (in the case of Toll, the kinase is not integral to the receptor). It also shares the Toll /interleukin-1 (IL-1) receptor (TIR) domain with several plant NLRs (fig. S2). Thus, the discovery of a role for Toll in the innate immune response provided a structural link between sensors used by plants and animals to detect infection.

Toll does not serve as a receptor for any known molecule of fungal origin, nor do eight of the nine Toll paralogs known in *Drosophila* (all of them save Toll itself) have any immune function whatsoever. Instead, Toll responds to Spaetzle, which is cleaved from an endogenous protein as a result of infection. This recognition leads to activation of Pelle and to signals that culminate in the production of antimicrobial peptides as well as hundreds of other proteins, most of unknown function (Fig. 1) (*9*).

A second sensing pathway in *Drosophila* detects Gram-negative bacteria. The Imd pathway shows striking similarities to the mammalian tumor necrosis factor (TNF) signaling pathway (Fig. 1) (*9*). Mammalian TLRs trigger TNF release, and TNF signals via a pair of receptors represented on most cells. Thus, two separate ancestral pathways may have been passed to insects but became fused together in mammals, or conversely, a single ancestral pathway was split in insects but retained intact in mammals. Notably, unlike its human counterpart receptor-interacting protein (RIP), *Drosophila* Imd lacks a kinase domain. To date, no kinases have been found to associate with the peptidoglycan receptor complex PGRP-LC, which operates as the transmembrane sensor for the Imd pathway.

The determination that Toll has an immune function in *Drosophila* immediately raised questions as to whether mammalian homologs, first reported in 1994 (*10*, *11*), might have a similar function. Although transfection-mediated overexpression of truncated, chimeric versions of one human TLR induced NF-κB activation in mammalian cells (*12*), no conclusions regarding the specificity of the receptor could be drawn on this basis; indeed it remained uncertain whether it responded to a microbial ligand, to an endogenous ligand, or was actually involved in infection at all. Unbiased genetic research revealed the actual function of this receptor and pointed to a specific role for TLRs in microbe sensing.

Mammalian host defense. In mammals, the identification of microbial inducers of innate immune responses, equivalent to the elicitors of the plant world, long preceded the discovery of the specific receptors that detect infection. The groundwork for receptor discovery was laid as early as the 1890s, when heat-stable molecules of microbial origin were shown to induce fever and shock in the mammalian host. Foremost among the inducers was endotoxin (LPS), represented in most Gram-negative bacteria (*13*). Widely known for its ability to induce septic shock, LPS is perhaps the most powerful elicitor of inflammation known in mammals but is not unique in a qualitative sense. Lipopeptides, double-stranded RNA, microbial DNA, flagellin, and other molecules of microbial origin elicit inflammatory responses similar to those provoked by LPS. The identification of the receptors for these molecules was the central challenge in the field of animal innate immunity.

In mammals, the cytokine response (and particularly TNF production) was taken as an indicator of a biological response to LPS. The existence of a nonredundant LPS receptor was strongly suggested by two spontaneous mouse mutations affecting a locus known as *Lps*. Both mutations rendered mice insensitive to LPS and highly susceptible to Gram-negative infection. The mutations were positionally cloned in 1998, revealing that these strains carried either a missense error in *Tlr4* or deletion of the entire locus (*14*). Genetic data predicted that LPS must directly interact with TLR4, in that certain isoforms of LPS were species-dependent in their stimulatory effects (*15*, *16*). Later TLR4 was found to contact LPS in conjunction with MD-2, a secreted host protein with a hydrophobic pocket into which most of the LPS lipid chains become inserted (*17*). An essential contribution to LPS sensing is also made by CD14, an LRR protein that facilitates engagement of LPS by the TLR4/MD-2 complex, and is absolutely required for the detection of highly glycosylated (smooth) LPS.

Twelve mouse TLRs and ten human TLRs are now recognized, and most respond to infection, each detecting a circumscribed collection of molecules of microbial origin. Mutations that abolish the function of individual TLRs cause selective susceptibility to a certain spectrum of microbes; mutations that prevent all TLR signaling cause severe and general immunodeficiency (*18*). Nucleic acid–sensing TLRs are positioned chiefly within endosomes; those that detect other components of microbes are located mainly at the cell surface, although they are subject to internalization, as are some plant sensors such as the *Arabidopsis* FLS2 receptor (*19*). TLRs lack kinase domains and signal via cytoplasmic TIR domains that recruit one or more adaptor proteins (MyD88, TICAM1, TRAM, or TIRAP) to propagate signaling (Fig. 1).

In addition to the TLRs, intracellular sensors of the retinoic acid-inducible gene I (RIG-I)–like helicase family (RIG-I, Mda5, and Lgp2) detect single- and double-stranded RNA and the 5′ triphosphate moiety of viral RNA. C-type lectins, including dectin-1 and DC-SIGN, as well as eIF2α kinases such as double-stranded RNA-dependent protein kinase (PKR) and general control nonderepressible 2 (GCN2) kinase, are known to sense microbial carbohydrates and viral nucleic acids, respectively. Inflammasomes also detect and respond to some pathogens and danger signals (among them asbestos, silica, and nigericin) often in a subsidiary, TLR-dependent manner. The cores of these inflammasomes are formed by intracellular proteins of the NOD-like receptor (NLR) family, including Nlrp1, Nlrp3, IPAF, and AIM2. NLR proteins mediate apoptotic and inflammatory responses. The NLR proteins are structurally similar to plant NLR proteins but do not carry TIR domains, which are apparently reserved for signaling by TLRs or IL-1, IL-18, or IL-33, either at the cell surface or within endosomes. In contrast to the animal NLR proteins, none of the plant NLRs has been demonstrated to bind conserved microbial signatures, nor do they associate with non-RD kinases, suggesting a distinct mode of activation for these proteins (*4*).

As discussed in recent reviews, successful pathogens have evolved countermeasures to host sensor-mediated immunity, for example, delivering inhibitors of the immune response directly into the plant cytoplasm via the TTSS (*8*). In animals, TTSS-mediated delivery of proteins with TIR domain structures is known (*20*). Poxviruses encode proteins with TIR domain facsimiles as well (*21*). These findings attest to the universal importance of the innate immune response apparatus and its efficacy in combating infection.

Comparisons Between Animals and Plants: What Two Billion Years of Evolution Has Changed and What It Hasn't

We now know that plants and animals respond to microbial signature molecules using analo-

gous regulatory modules, which likely came about as a consequence of convergent evolution (fig. S2) (*22*). For example, many plant, fly, and mammalian host sensors, including XA21, FLS2, EFR, Toll, and the TLRs, use the LRR domain as their ligand recognition and binding surface. LRR proteins are among the most avid binding reagents found in nature, used in both plants and animals for the engagement of proteins, lipids, glycans, and nucleic acids (*18*). Undoubtedly, the ability of LRRs to engage almost any type of molecule led to their selection as molecular building blocks in recombinatorial adaptive immune receptors in jawless fish (*23*), whereas other vertebrates used the immunoglobulin fold for the same purpose. The three-dimensional structures of several TLR extracellular domains (TLR3, TLR4, TLR2/6, and TLR1/2) suggest that all of these sensors share similar shapes (fig. S2).

Similarly, the non-RD kinase motif has been recruited by both plants and animals to transduce the innate immune response. Mutagenesis of the kinase domains of RIP1, RIP2, RIP4, IL-1 receptor-associated kinase (IRAK1), and XA21 demonstrate that kinase activity is at least partially dispensable for the innate immune response (see SOM). This is a departure from the conventional role of kinases in signaling, and in the cases of IRAK1, XA21, and RIP2, evidence suggests that these non-RD kinases function partly as phosphorylation-mediated scaffold proteins, as distinct from enzymes that mediate signaling through a phospho-relay cascade. Thus, it appears that plant and animal receptors that associate with non-RD kinases or carry the non-RD domain integral to the receptor serve as host sensors per se. In contrast, RD kinases regulate nonimmune responses or serve as coregulators of the non-RD host sensors and do not bind microbial signatures on their own.

In animals, the MyD88/IRAK1/IRAK4 complex associates with the RING finger ubiquitin ligase TNF receptor-associated factor 6 (TRAF6), which autoubiquitinates and also ubiquitinates other proteins to propagate TLR signaling. Similarly, XB3, a plant RING finger ubiquitin ligase, transduces XA21-mediated innate immunity (see SOM).

Plants do not have NF-κB–related transcription factors. Instead, the rice and *Arabidopsis* innate immune responses rely on WRKY-related transcription factors, which do not exist in animals. The WRKY motif is often accompanied by zinc finger and leucine zipper motifs, and as WRKY factor regulation is dependent on mitogen-activated protein kinase cascades in *Arabidopsis* (Fig. 1), WRKY and activator protein 1 (AP1) might be considered analogous.

Host sensor–mediated immune responses are essential for innate immunity in both plants and animals, but sustained or highly induced immune responses can be harmful. Thus, negative regulation of these pathways is critical. In animals, negative regulators act at multiple levels within TLR signaling cascades (*24*). Little is yet known about negative regulation of plant innate immunity, although one important class of negative regulators is the Ser/Thr protein phosphatase 2Cs (PP2Cs) (Fig. 1). Another important control of innate immune responses in both plants and animals is by endoplasmic reticulum (ER)–resident chaperones, which are required for TLR2, TLR4, TLR5, TLR7, TLR9, EFR, and XA21 biogenesis (Fig. 1).

Outlook

The lineages of humans and mice diverged 60 to 120 Ma, monocots and dicots about 170 to 235 Ma, insects and mammals >640 Ma, and plants and animals perhaps one billion years ago. If evolution is depicted as a tree, and extant species as terminal leaves on that tree, we must acknowledge that we have examined only a few of those leaves, gaining only a fragmentary impression of what is and what once was. As sequencing methodology advances, we will almost surely see that some species emphasize specific mechanisms of resistance to the relative exclusion of others. Witness *Drosophila* with its single immunologically active Toll receptor, *Arabidopsis* with its dozens of host sensors, and rice with its hundreds (*4*). Only recently, we were surprised to discover the independent evolution of a system of recombinatorial receptors mediating adaptive immunity in the jawless fishes (*25*) and the presence of a predicted microbial sensor in wheat with a structure that does not appear in rice (last common ancestor: a mere 50 to 70 Ma) (*26*). Many similar surprises likely await the examination of other "leaves."

In the future, researchers will increasingly focus on harnessing basic knowledge about host sensors to advance plant and animal health. A diverse array of conserved signatures from pathogenic microbes will likely be discovered. Many of these will almost certainly act as binding partners for the large class of predicted orphan host sensors present in agronomically important crops (*4*). Some will likely serve as new drug targets to control deadly groups of bacteria for which there are currently no effective treatments (*27*). Characterization of new host sensors will pave the way to interspecific and intergeneric transfer between plants of engineered receptors that confer resistance to a variety of pathogens. The effectiveness of this approach has already been demonstrated by the transfer of Xa21 and engineered derivatives to cultivated rice varieties (*3*), of a stripe rust resistance gene to cultivated wheat varieties (*26*), and of *Arabidopsis* EFR to tobacco and tomato (see SOM). In vertebrates as well, there may be room to engineer resistance. Adult chickens are remarkably indifferent to LPS. Would they be more sensitive to it and better able to resist Gram-negative infection if they expressed the mammalian version of TLR4? Are some microbes pathogenic to humans because they have managed to evade detection by human TLRs? Other manipulations may be imagined now that some of the essential building blocks of immunity have been elucidated.

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28. This paper is dedicated to Julius Rothstein (1830–1899) and his wife, Fanny Rothstein née Frank (1834–1911), the great, great grandparents and last common ancestors of the authors.

Supporting Online Material

www.sciencemag.org/cgi/content/full/330/6007/1061/DC1
SOM Text
Figs. S1 and S2
References

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