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Plant and Animal Pathogen Recognition Receptors Signal through Non-RD Kinases

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Plants and animals mediate early steps of the innate immune response through pathogen recognition receptors (PRRs). PRRs commonly associate with or contain members of a monophyletic group of kinases called the interleukin-1 receptor-associated kinase (IRAK) family that include *Drosophila* Pelle, human IRAKs, rice XA21 and *Arabidopsis* FLS2. In mammals, PRRs can also associate with members of the receptor-interacting protein (RIP) kinase family, distant relatives to the IRAK family. Some IRAK and RIP family kinases fall into a small functional class of kinases termed non-RD, many of which do not autophosphorylate the activation loop. We surveyed the yeast, fly, worm, human, *Arabidopsis,* and rice kinomes (3,723 kinases) and found that despite the small number of non-RD kinases in these genomes (9%–29%), 12 of 15 kinases known or predicted to function in PRR signaling fall into the non-RD class. These data indicate that kinases associated with PRRs can largely be predicted by the lack of a single conserved residue and reveal new potential plant PRR subfamilies.

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Introduction

Animal and plant innate immune systems use a set of defined pathogen recognition receptors (PRRs), also called pattern recognition receptors, that function as both cell surface and cytoplasmic pathogen surveillance proteins. These receptors provide a first line of defense against pathogen attack and rapidly activate defense signaling pathways following infection (reviewed in [1]). They play a crucial role in the switch from innate to adaptive immunity in mammals [2–4], and the signaling cascades initiated by PRRs are implicated in a number of human diseases [5–7]. PRR receptors and components of their signaling pathways share remarkable similarities in mammals, plants, and invertebrates. For example, a monophyletic family of serine-threonine kinase domains, the interleukin-1 receptor-associated kinase (IRAK) family (also known as pelle/RLK), including human IRAKs, *Drosophila* Pelle, and the large family of plant receptor kinases [8–10], either associate with PRRs or are present as intracellular domains of the receptor themselves. These are the first kinases in a kinase signaling cascade that leads, among other known functions, to the initiation of cellular defense responses. A graphic representation of some of the PRRs and PRR-associated kinases that function in innate immune systems in plants, *Drosophila,* and animals is shown in Figure 1.

In animals, recognition of pathogens at the cell surface is largely carried out by members of the Toll-like receptor (TLR) family of PRRs [3,11]. Activation of TLR-1, 2, 4, 5, or 6 triggers a well-characterized signaling pathway that activates nuclear factor-kappa B (NF-κB) transcription factors and leads to a core set of defense responses. In mammals, TLRs associate with IRAK kinases [1] as well as the IRAK related receptor interacting-protein (RIP) kinases [12,13] through adaptor proteins. While less is known about RIP kinases, IRAK1 becomes activated and rapidly phosphorylated upon pathogen recognition [14]. Activation triggers a downstream signaling pathway that has been well characterized [1,15]. A similar pathway is found in *Drosophila,* in which the activation of TLR receptors stimulates the recruitment of the IRAK family kinase Pelle [16].

Plants lack TLRs. Instead, extracellular pathogen recognition is mediated by two known classes of PRRs that lack intracellular kinase domains, serine-threonine receptor kinases and receptor-like proteins (RLPs). Some of the best-studied plant PRRs are the receptor kinases FLS2 [17] and XA21 [18] and the RLPs Cf-9 [19] and XA21D [20]. The signaling pathways of FLS2 and XA21 have been partially characterized [21,22].

Mammals contain at least three classes of intracellular PRRs: TLRs [11], nucleotide oligomerization domain (NOD) receptors [23–25], and two kinases, protein kinase R (PKR) [26] and RNAseL [27], that directly or indirectly detect virus-derived double-stranded RNA (dsRNA). PKR and RNAseL defend against virus attack by shutting down host cell transcriptional machinery and stimulating interferon-mediated innate immune responses that are, in part, controlled by NF-κB [28]. While similar to their cell surface counterparts, TLR-3, 7, 8, and 9 are intracellular receptors that recognize viruses, bacteria, and intracellular parasites [11]. TLR-3, like

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Abbreviations: ACF, alternative catalytic function; dsRNA, double-stranded RNA; IRAK, interleukin-1 receptor-associated kinase; LRR, leucine-rich receptor; NBS-LRR, nucleotide binding site leucine-rich receptor; NF-κB, nuclear factor-kappa B; NOD, nucleotide oligomerization domain; PKR, protein kinase R; PRR, pathogen recognition receptor; RIP, receptor-interacting protein; RLCK, receptor-like cytoplasmic kinase; RLP, receptor-like proteins; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor

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Synopsis

Animal and plant innate immune systems use a set of similar receptors to recognize disease-causing microbes. These receptors function in pathogen surveillance and are located either at the cell surface or inside the cell. They provide a first line of defense against pathogen attack and rapidly activate defense signaling pathways following infection. Key to their ability to respond to pathogens, a closely related family of proteins called kinases either associate with these receptors or are present as part of the receptors themselves. These kinases face two challenges. The signals must be carefully modulated, as misregulation can result in disease and overall poor health. Second, these signaling systems must be resilient to attempts by pathogens to interfere with and block defense responses. The researchers have found that the kinases that are linked to pathogen receptors and initiate innate immune responses contain an alteration within a critical functional domain of the kinase that is not commonly found in similar kinases that control nondefense pathways. While the exact impact these changes have on kinase function is unclear, these findings provide insight into how these kinases may have evolved to compensate for the unique challenges they face. Moreover, they provide a predictive tool for identifying new candidate kinases that control innate immune responses.

PKR and RNAseL, responds to viral dsRNA and can activate interferon-mediated pathways [29]. A more recently described class of PRRs is the NOD receptors, which are known to detect bacterial pathogens. RIP2 kinase (also known as RICK and CARDIAC) interacts directly with NOD1 [23] and NOD2 [24] and is required for the activation of NF-κB [25]. This pathway also requires IRAK1; however, the role of IRAK1 in NOD receptor signaling is unknown [25].

Intracellular recognition of plant pathogens is carried out by NOD receptors (more commonly referred to as nucleotide binding site leucine-rich receptors [NBS-LRRs]) that have been long known to confer strain-specific resistance to numerous viral, bacterial, fungal, and nematode pathogens. Although little is known about NBS-LRR signaling complexes, they are not known to directly associate with and/or signal through kinases. There is a growing body of evidence that many NBS-LRRs indirectly detect pathogens and respond to alterations of host proteins caused by pathogen effectors [30].

Little is known about how IRAK and RIP family kinases are regulated and stimulated by their cognate receptors. In general, kinase regulation can be achieved through a wide variety of mechanisms and relies on specific phosphorylation events. In response to stimulation, phosphorylation of regulatory domains both within and outside of the kinase domain can alter kinase activity, create substrate-binding sites or disrupt inhibitory protein-protein interactions [31]. In many cases a combination of these mechanisms are employed to tightly regulate kinase activation and signaling. Among these varied strategies, autophosphorylation of a regulatory region termed the activation loop is critical to most kinases [32]. The activation loop, which comprises a short stretch of residues between kinase subdomains VII and VIII, typically resides in close proximity to the kinase active site and has at least two known functions. In some kinases the activation loop blocks substrate access to the active site in the kinase-inactive state and, upon phosphorylation, conformational changes expose the active site [32]. In addition, the activation loop sometimes serves to activate kinases by increasing phosphotransfer efficiency [32].

Kinases that are regulated by activation loop phosphorylation typically carry a conserved arginine (R) immediately preceding the invariant aspartate in subdomain VI required for catalytic activity [31]. The R is central to cluster of positively charged residues that inhibit catalysis by the adjacent negatively charged aspartate (D) located within the active site and becomes neutralized by contacts with a negatively charged phosphoamino acid(s) in the activation loop. This charge neutralization is essential for proper orientation of the highly conserved D in subdomain VII (DFG motif) that facilitates phosphotransfer [33]. Kinases that are regulated through this mechanism are commonly referred to as RD kinases and are found in bacteria, fungi, plants, and animals.

Conversely, a smaller number of kinases can be grouped into a class referred to as non-RD. Non-RD kinases lack the conserved R in kinase subdomain VI. It has been observed that some non-RD kinases do not autophosphorylate the activation loop and are either constitutively active or regulated through alternative mechanisms [31]. The most notable exceptions fall within the DYRK family of non-RD kinases, which autophosphorylate an activation loop tyrosine residue via an intermediate form that occurs only during translational synthesis [34]. However, in other non-RD kinases the lack of activation loop phosphorylation is consistent with the apparent lack of need for charge neutralization and removal of catalytic inhibition [31]. Despite this commonality, how changes to the RD motif impact kinase function in most cases remains unclear.

The rice PRR XA21 falls into the non-RD class of kinases and is not autophosphorylated in the activation loop in vitro [35]. Likewise, we observed that some related animal kinases, such as IRAK1 and Pelle, were also non-RD. This cross-kingdom correlation prompted us to perform a genome-wide comparison of plant and animal kinases to determine if non-RD motifs are common in kinases associated with pathogen recognition. We surveyed the complement of non-RD kinases among the yeast, fly, worm, human, *Arabidopsis,* and rice genomes and found that, while less than 20% of all kinases are non-RD, all six plant PRR kinases and six of nine animal PRR-associated cytoplasmic kinases that are known or predicted to transmit PRR signals are non-RD. Furthermore, plant non-RD receptor kinases that function as PRRs show evolutionary expansion patterns characteristic of disease resistance genes.

Results

Non-RD Kinases Are Evolutionarily and Functionally Conserved

We first analyzed the overall distribution, conservation, and functional significance of changes to the RD motif. Kinases were classified based on the presence (RD) or absence (non-RD) of the highly conserved R. In addition, kinases lacking one or more of three highly conserved residues thought to be required for catalytic activity were classified as alternative catalytic function (ACF) (Tables S1, S2, and S3). Together, the yeast, fly, worm, human, *Arabidopsis,* and rice kinomes are composed of 3,723 kinases. (Detailed information regarding our unpublished rice kinome can be found at

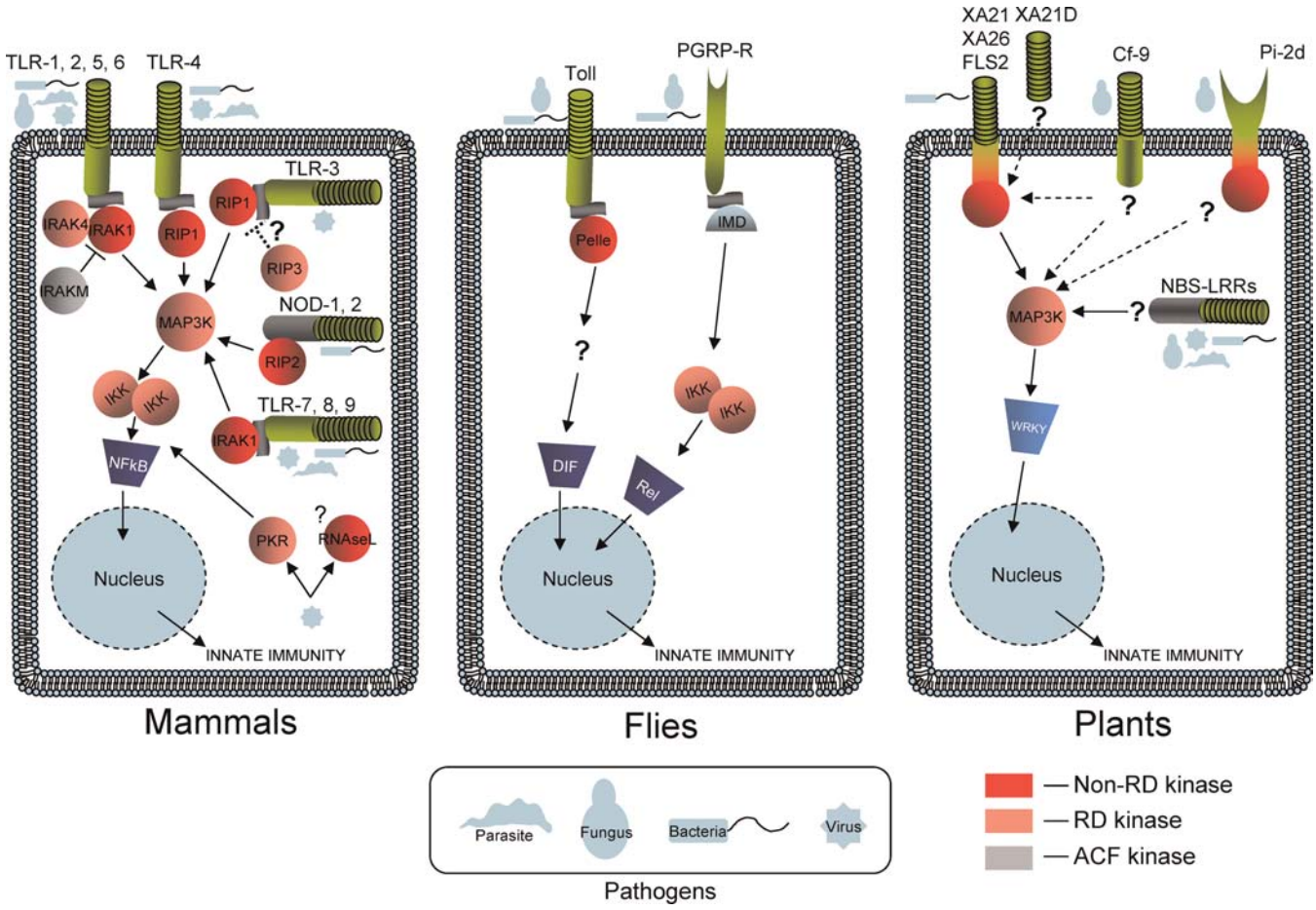

Figure 1. The Role of Kinases in Known PRR-Mediated Pathways

Summary of known innate immunity pathways in humans [1,3,11,23,24,26,90], flies [16,], and plants [17,18,19,20]. Kinases are represented by circles. Fly IMD is orthologous to human RIP1 but lacks the kinase domain. Non-RD kinases associate with PRR complexes or, in the case of plants, are intracellular domains of membrane-spanning receptors. Activation of non-RD kinases leads to innate immune signaling via mitogen-activated protein kinase pathways and ultimately stimulates defense-specific transcription factors (blue) in the NF-κB/Rel family or in the case of plant FLS2, the WRKY family.
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http://rkd.ucdavis.edu.) Most kinomes contained relatively few non-RD kinases (9%–16%) with the exception of rice, which had an unusually high number (29%) (Table 1). Of all non-RD kinases, 62% contained a cysteine or glycine, while another 20% contained the hydrophobic amino acids phenylalanine and leucine in substitution of the highly conserved R (Figure 2 and Table S4). These substitutions are predicted to lead to the loss of positive charge and potentially alter kinase regulatory and/or catalytic mechanisms. This limited range of amino acid substitutions suggests that diverse non-RD kinases have adopted similar modifications of the RD motif.

In all kinomes, non-RD kinases were distributed among six of the seven kinase groups. The vast majority were found in phylogenetically distinct clusters within the CAMK (calcium/calmodulin-dependent protein kinase), CMGC (containing CDK, MAPK, GSK3, CLK families), CK1 (casein kinase 1), and

Table 1. Percentage of Kinase Classes in Eukaryotic Genomes

Organism	Kinome size[a]	% RD	% Non-RD	% ACF	Source of Kinase Sequences
Yeast	115	82% (94)	14% (16)	4% (5)	http://www.kinase.comhttp://www.kinase.com
Fly	227	69% (157)	16% (36)	15% (34)	http://www.kinase.com
Worm	430	67% (288)	9% (39)	24% (104)	http://www.kinase.com
Human	491	75% (368)	14% (68)	11% (55)	http://www.kinase.com
Arabidopsis	1,027	70% (720)	10% (104)	20% (203)	http://hodgkin.mbu.iisc.emet.in/~kinghttp://hodgkin.mbu.iisc.ernet.in/~king/
Rice	1,429	56% (805)	29% (419)	14% (205)	http://rkd.davis.eduhttp://rkd.ucdavis.edu

Total number of kinases in each class is shown in parentheses.

[a]Kinome sizes reflect the total number of kinase domains, with the exception of atypical kinases, which were not included in this analysis. Some worm, fly, and human kinases contain two kinase domains. These second kinase domains are indicated with _b after their names in Tables S1, S2, and S3.

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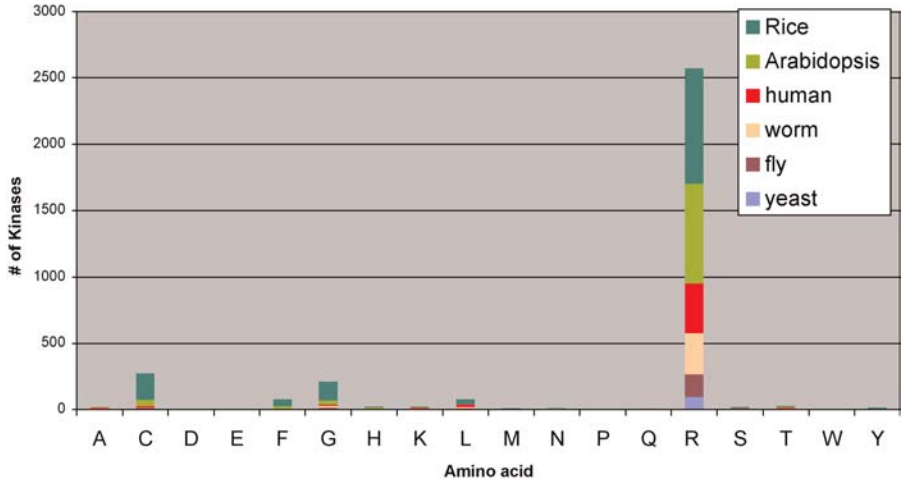

Figure 2. Conservation of Arginine in Kinase Subdomain VI (RD) Motif

The frequencies of amino acid residues occurring in each kinome are shown. Single-letter codes for amino acids are listed on the x-axis. The y-axis values represent the number of kinases containing each residue. Non-RD kinases account for 18% of all kinases, with rice containing the vast majority.
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TKL (tyrosine kinase-like) groups. This is illustrated on the human kinome phylogenetic tree (Figure 3) [36]. Of non-RD kinases, 518 (76%) of 681 were members of conserved kinase families found in all five multicellular eukaryotic genomes surveyed, while 50 (7%) of the 681 were unique to a single kinome. Kinases conserved in at least five kinomes are listed in Table 2, and a complete list of all non-RD kinases can be found in Table S5. Few examples were found of non-RD kinases containing RD counterparts in another organism, suggesting a high degree of evolutionary and functional conservation (Table S5). A small number of non-RD kinases in plants and invertebrates have known functions; however, substantial information is available regarding the functions of human non-RD kinases (Table 3). Not surprisingly, some non-RD kinases, such as MLCK (myosin light chain kinase) [37] have diverse roles and function in multiple cellular processes. Despite the diversity of functions, 43 of 50 non-RD kinases had known roles in innate immunity, apoptosis, and/or cell cycle control. Markedly infrequent were functions associated with growth, development, cell-to-cell communication, and general stress responses. The finding that non-RD kinases function across a relatively confined set of pathways suggests that these altered kinase regulatory and/or catalytic mechanisms are not widely utilized in cell signaling pathways and may have specifically evolved to control a smaller subset of cellular processes.

Kinases That Control Early Events of Innate Immunity Signaling in Animals Are Non-RD

To investigate the role of non-RD kinases in animal innate immune systems, we analyzed the known functions of all kinases that lead to the activation of NF-κB in mammals or the orthologous DIF/Relish transcription factors in *Drosophila*. A complete list of all animal PRR kinases with known functions is shown in Table 4. We found that six of nine kinases known or predicted to associate with PRRs and relay PRR signals were non-RD. Five non-RD kinases in the IRAK and RIP families and one kinase in the "Other" group were found to play prominent roles in early steps of innate immunity pathways (Figure 1, Table 4). These include IRAK1 [38], Pelle [39], RIP1 [13], and RIP2 [12], which directly associate with NOD and/or TLR type PRRs and play primary roles in signaling (see [40] and [41] for reviews on IRAK and RIP kinases). One additional RIP family member, RIP4, has been shown to activate NF-κB when overexpressed [42]. The sixth non-RD kinase, RNAseL, falls into the group "Other" and indirectly responds to viral dsRNA [27]. The three exceptions, IRAK2 (an ACF kinase), IRAK4 (RD) and PKR (RD), have unique or limited roles in PRR signaling. IRAK2 and IRAK4 have a limited ability to propagate PRR signals and/or need non-RD kinases to function. For example, IRAK4 is capable of only weak NF-κB activation in the absence of IRAK1 and is thought to recruit IRAK1 to the PRR complex, where IRAK1 becomes activated and leaves the receptor complex to facilitate downstream signaling [43,44]. IRAK2 [45] is catalytically inactive and mediates NF-κB activation via a MAL/TIRAP (Myd88 adaptor-like protein) that functions in a cell-specific manner to partially control TLR-4 responses [46]. Unlike its human homolog, mouse IRAK2 does not appear to play a role in NF-κB activation [47], and two splice variants of mouse *Irak2* were found to function as negative regulators of innate immune responses [48]. Similarly, PKR, like RNAseL, responds to viral dsRNA; however, PKR kinase activity is not needed to trigger NF-κB activation, suggesting that this function of PKR may be mediated by other proteins such as a non-RD kinase [49]. Of the remaining four IRAK and RIP kinases in humans, three have unknown functions (RIP5 and 6 [non-RD] and RIP7 [RD]) [41] and two act as negative regulators of TLR signaling (IRAK3/IRAKM [ACF] and RIP3 [RD]) [50–52]. Taken together, all NOD- and TLR-type animal PRRs appear to associate with and utilize non-RD IRAK and/or RIP kinases to activate NF-κB pathways, whereas RD and ACF kinases appear to play ancillary roles in signaling.

Other Receptor-Mediated Pathways Leading to NF-κB Activation in Humans Also Utilize Non-RD Kinases

A number of non-PRR receptor-mediated pathways are also known to activate NF-κB in humans, including the tumor necrosis factor receptor (TNFR) family of receptors, which

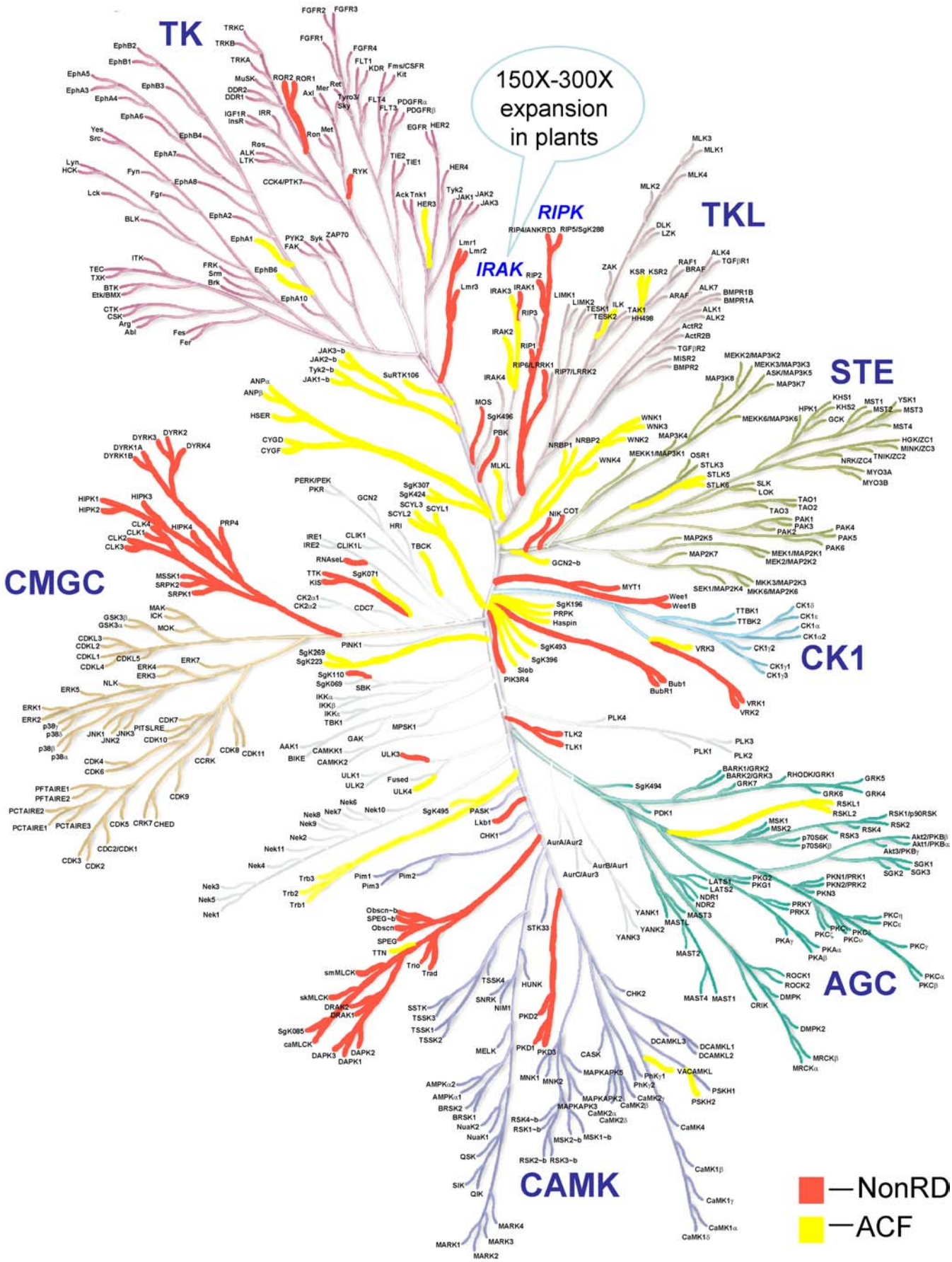
TK
TKL
STE
CMGC
CK1
AGC
CAMK
RIPK
IRAK
150X-300X expansion in plants
NonRD
ACF

Figure 3. Distribution of Non-RD Kinases within the Human Kinome

A reproduction of the human kinome tree is shown [36]. Kinome dendrogram was reproduced courtesy of Science and Cell Signaling Technology (http://www.cellsignal.com). Names of kinase groups are indicated: AGC (PKA, PKG, and PKC kinases), CMGC (containing CDK, MAPK, GSK3, CLK families), CAMK (calcium/calmodulin-dependent protein kinase), CK1 (casein kinase 1), TK (tyrosine kinases), TKL (tyrosine kinase-like; includes MLKs [mixed lineage kinases], transforming growth factor-β receptor kinases, and Raf kinases), STE (homologs of yeast sterile 7, sterile 11, and sterile 20 kinases). Branches leading to non-RD kinases and ACF kinases are colored red and yellow, respectively. All other branches contain RD kinases. The IRAK and RIP families are indicated above their respective clades. The IRAK family is greatly expanded in *Arabidopsis* (610) and rice (1,068). Most non-RD kinases are present in phylogenetically linked clusters within the TKL, CAMK, CMGC, and CK1 groups.

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play critical roles in apoptotic signaling as well as in innate and adaptive immune responses (reviewed in [53]), and the epidermal growth factor receptor [54]. The non-RD kinases IRAK1, RIP1, COT/Tpl-2, and death-associated protein kinase associate with TNFR complexes via TNFR-associated factor, TNFR-associated death domain, and Fas-associated death domain adaptor proteins [55–57]. Similarly, the epidermal growth factor receptor associates with the non-RD kinases RIP1 and NF-κB-inducing kinase; however, unlike TNFRs, it does not appear to require TNFR-associated death domain or Fas-associated death domain adaptor proteins [54]. Most RIP, IRAK, and death-associated protein kinases, with the exception of RIP3–7, share a common domain in the death domain superfamily that includes the death domain, death effector domain, and caspase recruitment domain [58]. These domains mediate protein-protein interactions and link these kinases to receptors and receptor-adaptor proteins that also contain death domains. Therefore, in addition to known PRR-mediated innate immunity pathways, other receptor-mediated pathways leading to NF-κB activation in humans also utilize non-RD kinases.

Non-RD Plant Receptor Kinases Function in Innate Immunity

A survey of IRAK family receptor kinases in plants revealed a perfect correlation between those that function in innate immunity and non-RD motifs (Table 5). Out of 38 receptor kinases with known or implied functions, six are known or presumed to function in disease resistance and act as PRRs. All six fall into the non-RD class. These include the XA21 [18],

Table 2. Cross-Kingdom Conservation of Non-RD Kinases

Group	Family	Yeast	Fly	Worm	Human	*Arabidopsis*	Rice	Function(s)	Reference(s)
CMGC	DYRK	Yak1	DYRK3 Smi35A Mnb	Mbk1 Mbk2	DYRK1A DYRK1B DYRK2 DYRK3 DYRK4	At5g35980 At2g40120 At3g17750 At1g73450 At1g73460	9630.04961 9630.m04611 9629.m06068 9633.m03633 9631.m04986	Regulation of cell growth	[91]
CMGC	DYRK/PRP4	—	CG7028	F22D6.5	PRP4	At3g25840 At1g13350 At3g53640	9640.m04369 9631.m04905 9633.m03633 9629.m06068	RNA splicing	[92]
CMGC	CLK	KNS1	DOA	E02H4.3	CLK1 CLK2 CLK3 CLK4	AFC1 AFC2 AFC3	9629.m06124 9640.m04396 9629.m03900	RNA splicing	[93]
CMGC	SRPK	SKY1p	SRPK1 CG8565 CK01209—best	SPK-1	SRPK1 SRPK2 MSSK1	SRPK1 SRPK2 SRPK3 SRPK4	9631.m05272 9635.m02814	RNA splicing	[94]
CMGC	TTK	MPS1	CG7643	C24g7.5 F12F3.2 UNC89	TTK	AT1g77720	9631.m04854	Cell cycle control	[95]
Other	Bub	Bub1	CG14030	R06C7.8 BubR1	Bub1	At2g20635[a]	Os07g32480	Cell cycle control	[96]
Other	Wee	Swe1	Myt1 Wee1 Wee1B	Wee1.1 Wee1.3	Myt1 Wee1	Wee1	9630.m00332	Cell cycle control	[97]
Other	VPS15	VPS15	CG9746	ZK930.1	PIK3R4	At4g29380	Os02g55340	Vacuolar sorting	[98]
Other	Bud32	Bud32	CG10673	F52C12.2	PRPK	At5g26110	Os10g28640	Phosphorylates p53	[99]
Other	TLK	—	TLK	C07A9.3	TLK1 TLK2	Tousled	9631.m05290	Transcription	[100]
TKL	IRAK	—	Pelle	Pik-1	IRAK1 IRAK2[b] IRAK3[b] IRAK4[a]	609 kinases 47 non-RD 139 ACF	1,065 kinases 371 non-RD 162 ACF	Innate immunity, development, stress, symbiosis	[See Tables 3 and 4 for references]

[a]RD kinase

[b]ACF kinase

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Table 3. Functions of Human Non-RD Kinases

Kinase	Group	Family	Function	Reference(s)
IRAK1	TKL	IRAK	Innate immunity	[38]
RIP1, 2, 4	TKL	RIP	Innate immunity, apoptosis, cell stress	[41]
Lmr1, 2, 3	TK	Lmr	Apoptosis	[101]
cMOS	Other	MOS	Cell cycle control, apoptosis, differentiation	[102]
PBK/TOPK	Other	TOPK	Cell cycle control	[103]
NIK, COT	STE	STE—unique	Innate immunity	[104–106]
Bub1, BubR1	Other	Bub	Cell cycle control, apoptosis	[96]
VRK1, 2	CK1	Vrk	Regulates p53	[107]
PRPK	Other	Bud32	Cell cycle control, apoptosis	[99]
Wee1, Wee1B, Myt1	Other	Wee	Cell cycle control	[97]
CLK1, 2, 3, 4	CMGC	CLK	Regulation of RNA splicing	[93]
HIPK 1, 2, 3, 4	CMGC	CLK	Transcriptional regulation, apoptosis, growth suppression.	[108]
DYRK 1A, 1B, 2, 3, 4	CMGC	DYRK	Cell proliferation, development, cell cycle control	[109]
SRPK1, 2	CMGC	SRPK	RNA splicing	[110]
PKD1, 2, 3	CAMK	PKD	Golgi organization, plasma membrane directed transport, metastasis, immune responses, apoptosis, cell proliferation	[111]
Trio	CAMK	Trio	Regulation of actin cytoskeleton	[112]
DRAK1, 2	CAMK	DAPK	Apoptosis	[113]
DAPK1, 2, 3	CAMK	DAPK	Apoptosis	[114]
smMLCK, skMLCK	CAMK	MLCK	Muscle assembly, protein secretion, apoptosis	[37]
Lkb1	CAMK	CAMKL	Cell cycle control, apoptosis	[115]
TLK 1, 2	Other	Tlk	Cell cycle control	[100]
RNAseL	Other	Other—unique	Innate immunity, apoptosis, Translation initiation	[27,28]
TTK	Other	TTK	Cell cycle control	[95]
KIS	Other	Other—unique	Cell cycle control	[116]

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XA26 [59], and Pi-2d [X. Chen, unpublished data] resistance genes from rice, the FLS2 [17] and PR5K [60] genes from *Arabidopsis*, and wheat LRK10 [61], which is linked to a disease resistance locus. The remaining 32 receptor kinases are RD or ACF kinases that function in a wide variety of signaling pathways, including development, pollen recognition, steroid perception, stress/pathogen responses, and interactions with symbiotic organisms. Two RD kinases, ERECTA [62] and WAKL22 [63], have been found to incrementally contribute to pathogen resistance in addition to their known roles in development; however, these receptor kinases are not known to function as PRRs, and both ERECTA and WAKL22 were identified as components of quantitative trait loci. Therefore, although diverse IRAK family kinases control many cellular processes in plants, it is clear that as with animal systems, plant PRRs utilize non-RD kinases.

In contrast to animals, plant IRAK family cytoplasmic kinases are not known to associate with and directly relay PRR signals. Of the 22 receptor-like cytoplasmic kinase (RLCK) subfamilies in rice and *Arabidopsis* (Table S6), only

Table 4. List of Known and Predicted Animal PRR Kinases

	Name	Group	Family	Class	Organism	Role in PRR Signaling	References
Animal IRAK & RIP PRR Kinases	IRAK1	TKL	IRAK	Non-RD	Human	TLR1,3,5,6,7,8,9	[38]
	IRAK2	TKL	IRAK	ACF	Human	TLR4	[45]
	IRAK4	TKL	IRAK	RD	Human	TLR1,3,5,6	[43,44]
	Pelle	TKL	IRAK	Non-RD	Fly	Toll	[39]
	RIP1	TKL	RIP	Non-RD	Human	TLR3, TLR4	[13]
	RIP2	TKL	RIP	Non-RD	Human	NOD1,2	[12]
	RIP4	TKL	RIP	Non-RD	Human	Predicted	[42]
	RNAseL	Other	Other—unique	Non-RD	Human	Indirectly detects dsRNA	[27]
	PKR	Other	Other—unique	RD	Human	Binds dsRNA	[26]
Animal IRAK & RIP family members with alternative or unknown functions	IRAK3	TKL	IRAK	ACF	Human	Negative regulator TLR4, 9	[51]
	Pik-1	TKL	IRAK	Non-RD	Worm	Unknown	[86]
	RIP3	TKL	RIP	RD	Human	Negative regulator RIP1	[52]
	RIP5	TKL	RIP	Non-RD	Human	Unknown	
	RIP6	TKL	RIP	Non-RD	Human	Unknown	
	RIP7	TKL	RIP	RD	Human	Parkinson disease	[117,118]

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Table 5. Plant Non-RD Receptor Kinases Are Associated with Innate Immunity

Kinase	Class	Subfamily	Plant	Function(s)	Reference(s)
CRINKLY4	RD	CR4L	Maize	Surface formation of epidermis-related tissues	[119]
CHRK1	RD	DUF26 Ia	Tobacco	Plant development and cytokinin homeostasis	[120]
CRK5	RD	DUF26 Ib	*Arabidopsis*	Promotes leaf growth, cell death, and potentiates SA resistance	[121]
RLK4	RD	DUF26 Ib	*Arabidopsis*	SA inducible	[122]
LecRK	RD	L-LEC	Cotton	Cotton fiber development	[123]
SIRK	RD	LRR Ia	*Arabidopsis*	Senescence	[124]
SYMRK	RD	LRR Ic	*Medicago*, Lotus	Symbiotic interactions	[125,126]
BAK1/SERK3	RD	LRR II	*Arabidopsis*	Growth and development	[127]
SCM	RD	LRR V	*Arabidopsis*	Positional signaling in developing root epidermis	[128]
RKF1	RD	LRR VIII-2	*Arabidopsis*	Pollen-specific expression	[129]
Bri1/Systemin	RD	LRR Xb	*Arabidopsis*	Growth and development, wound response	[130,131]
BRL 1,3	RD	LRR Xb	*Arabidopsis*	Vascular differentiation	[132]
EMS1	RD	LRR Xb	*Arabidopsis*	Microspore development	[133]
PSK	RD	LRR Xb	Carrot	Growth and development	[134]
CLAVATA1	RD	LRR XI	*Arabidopsis*	Apical meristem proliferation-symbiotic interactions	[135]
Td1	RD	LRR XI	Maize	Male and female inflorescence development	[136]
FON1	RD	LRR XI	Rice	Regulation of floral organ number	[137]
HAESA/RLK5	RD	LRR XI	*Arabidopsis*	Controls floral organ abscission	[138]
ERECTA	RD	LRR XIIIb	*Arabidopsis*	Organ initiation and elongation	[139]
RPK1	RD	LRR XV	*Arabidopsis*	Regulator of abscisic acid early signaling	[140]
NFR1/LYK3, 4	RD	LysM I	Lotus, *Medicago*	Symbiotic interactions	[87,141]
LYK4	RD	LysM I	Lotus	Symbiotic interactions	[84]
NFR5/SYM10	RD	LysM II	Lotus, pea	Symbiotic interactions	[81]
PERK1	RD	PERK	*Brassica*	Wound inducible	[142]
SRK	RD	SD-1a	*Brassica*	Pollen self incompatibility	[143]
SFR/ARK	RD	SD-1a	*Brassica, Arabidopsis*	Induced by SA, development	[144]
WAK1	RD	WAKb	*Arabidopsis*	Plant survival after bacterial infection, aluminum tolerance	[145,146]
WAK4	RD	WAKb	*Arabidopsis*	Cell elongation	[147]
WAKL	RD	WAKL	*Arabidopsis*	Environmental stress response	[148]
PRK1	ACF	LRR III	*Petunia*	Microspore development	[149]
GhRLK1	ACF	LRR VI	Cotton	Cotton fiber development	[150]
IRK	ACF	LRR VIIa	*Arabidopsis*	Expressed in meristematic tissues	[151]
LRK10	non-RD	LRK10L-2	Wheat	Resistance to fungal pathogen	[61]
PR5K	non-RD	LRK10L-2	*Arabidopsis*	Extracellular antimicrobial domain	[60]
XA21	non-RD	LRR XII	Rice	Resistance to bacterial pathogens	[18]
Xa26	non-RD	LRR XII	Rice	Resistance to bacterial pathogens	[59]
FLS2	non-RD	LRR XII	*Arabidopsis*	Resistance to bacterial pathogens	[17]
Pi-d2	non-RD	SD-2b	Rice	Resistance to fungal pathogens	[X. Chen, unpublished data]

All plant receptor kinases with known or implied functions are listed.
SA, salicylic acid.
DOI: 10.1371/journal.ppat.0020002.t005

three kinases have been linked to pathogen recognition. PTO and PBS1 are RD kinases that function in concert with the NOD-like NBS-LRR receptors PRF and RPS5, respectively, and provide resistance against bacterial pathogens. Rather than functioning to relay PRR signals, PTO [64] and PBS1 [30] are thought to be the targets of bacterial effector proteins. A third RLCK, RPG1, contains dual kinase domains (one non-RD and one ACF) and functions in perception of a fungal pathogen [65]. To date, it is not known if RPG1 also requires NBS-LRR receptors to trigger innate immune responses or if RPG1 associates with an RLP. Although it has been repeatedly speculated that RLP-family PRRs that lack kinase domains, such as XA21D [20] or Cf-9 [19], transmit signals via receptor kinase or cytoplasmic kinase partners, such partners have not yet been confirmed. Potential candidates include those RLCK family kinases with non-RD domains. For example, the rice RLCK-OS2 subfamily, which is closely related to RPG1, contains single non-RD kinase domains and have unknown functions. RLCKs known to function in non-defense pathways were all found to be RD or ACF kinases (Table S7).

The Correlation between Non-RD Kinases in the IRAK Family and Innate Immunity Is Statistically Significant

IRAK family kinases control diverse cellular processes in humans, *Drosophila*, and plants and are monophyletic. These characteristics make this group amenable to statistical analysis of kinase function and RD motifs. We analyzed whether the correlation between non-RD kinases in the IRAK family and PRR signaling is statistically significant. Fisher's exact test was used to calculate the probability that the observed correlation can be explained by random associations (Table S7). In contrast to Chi-square analysis, Fisher's exact test accurately predicts probabilities in datasets containing values less than 5. Fifty-two IRAK family kinases were found to have known or implied functions; nine non-RD, seven ACF, and 36 RD. All nine non-RD kinases plus one RD and two ACF kinases were identified as PRRs or are known to associate with PRR complexes. The remaining 40 RD and ACF kinases had a wide variety of cellular and developmental functions. The resulting two-tailed p-value was less than 0.0001, indicating a very high likelihood that the distribution of non-RD IRAK kinases with respect to a role in

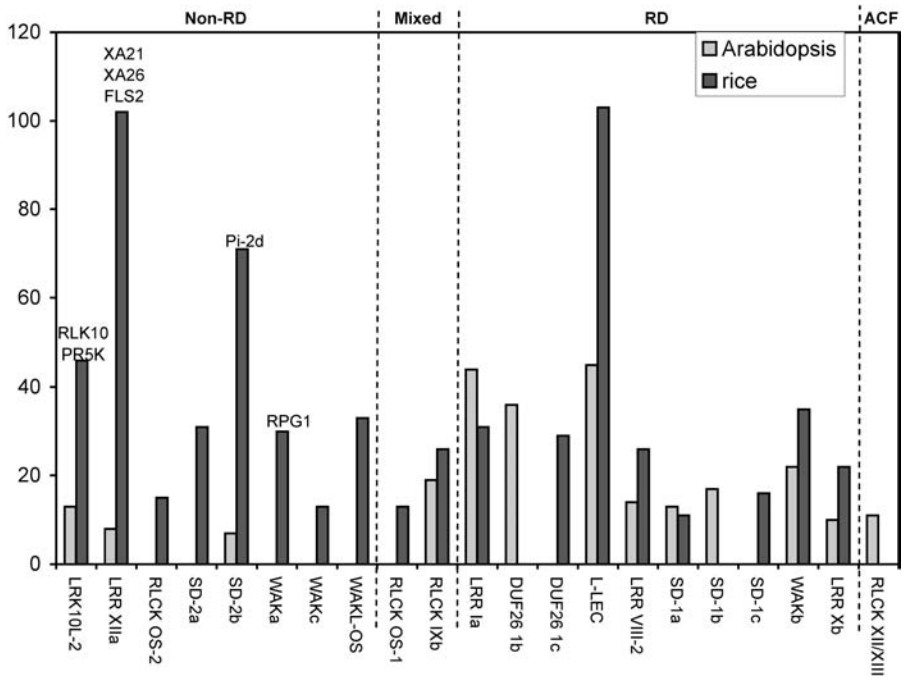

Figure 4. Expansion IRAK Subfamilies in Plants

Rice and *Arabidopsis* IRAK subfamilies that show evidence of recent expansion are listed on the x-axis. The number of kinases in each subfamily is indicated on the y-axis. Non-RD, mixed, RD, and ACF subfamilies are grouped and separated by dotted lines. Known disease-resistance genes are listed above corresponding subfamilies.

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PRR signaling is not random. Conversely, PRR kinases could not be predicted based on protein domain organization or phylogenetic relationships. A tree showing evolutionary relationships among IRAK kinases with known functions is shown in Table S7. Corroborative trees that include all IRAK kinases are listed in [9] and [10].

Expansion of Non-RD IRAK Subfamilies in Rice

The IRAK kinase family in plants consists of numerous receptor and cytoplasmic subfamilies that have been delineated based on phylogenetic analyses and organization of extracellular domains [9,10]. A comparative study of *Arabidopsis* and rice indicated that IRAK family kinases associated with defense responses have undergone lineage-specific expansions due to both tandem and large scale duplications within the genomes, in some cases resulting in large subfamilies (over 100 kinases) [10]. For example, the LRR XII subfamily that includes the PRRs XA21 and FLS2 contains over 100 members in rice and only eight in *Arabidopsis*. Similar expansions in plants have also been observed for the NBS-LRR class of PRRs [66]. In contrast, IRAK family kinases associated with development have remained largely unchanged since the last common ancestor of these two highly divergent plant species [10]. We predicted that, if non-RD kinases in plants function as PRRs, they should primarily fall into these expanded subfamilies. In contrast, we predicted that RD subfamilies not associated with innate immunity would tend to show distinct evolutionary patterns, since they are likely under different selection pressures.

Plant IRAK kinase subfamilies were designated as RD, non-RD, or ACF if at least 70% of the members fell into one class (Table S6). Using new data from the assembled Japonica rice genome, we analyzed the composition and chromosomal distribution of all rice and *Arabidopsis* IRAK kinase subfamilies and selected them based on the following criteria: at least eight members within the subfamily, lineage-specific expansion (i.e., expansion in rice and/or *Arabidopsis*), and at least one cluster of four or more closely related genes as defined by Holub (Tables S6, S8, and S9) [67]. Chromosomal distributions of all kinases were visualized using Genome Pixelizer [68]. The color-coded images are available in Figure S1 and at our Web site at: http://rkd.ucdavis.edu. The results are summarized in Figure 4.

In total, rice contains 456 more IRAK family kinases than *Arabidopsis,* and expanded non-RD kinase subfamilies accounted for 69% of this difference (313 of the 456). Expanded non-RD subfamilies included all known PRR kinases: XA21 [18], Xa26 [59], FLS2 [17], and Pi-2d [X. Chen, unpublished data], as well as the non-RD RLCK subfamily RLCK-OS2, which is most similar to the resistance gene product RPG1 [65]. Furthermore, all seven non-RD IRAK family receptor kinase subfamilies in rice, and two of three in *Arabidopsis,* show recent lineage specific expansions. This finding is consistent with the hypothesis that non-RD IRAK subfamilies in plants function in pathogen recognition and innate immunity.

Discussion

Most IRAK family kinases in animals have known roles in innate immunity. In plants, members of this very large family (in *Arabidopsis,* 600; in rice, 1,000) play roles in a wide variety of physiological, developmental, and stress pathways in addition to pathogen defense. Here we present evidence that

members of the IRAK family controlling innate immune responses can be distinguished from members that control nondefense pathways. We found a significant correlation between the absence of a highly conserved R residue in kinase subdomain VI (non-RD) and a role in the early events of innate immune signaling. These non-RD kinases include human IRAK, *Drosophila* Pelle, and the plant kinases XA21, XA26, FLS2, RLK10, PR5K, LRK10, RPG1, and PI-D2—all known or predicted to function as PRRs. Additionally, kinases with functions analogous to IRAKs, such as those in the RIP family, also commonly contain non-RD motifs.

Because less than 40 of the hundreds of identified plant receptor kinases have known or predicted functions, we took additional steps to determine the extent of the correlation between non-RD kinases and innate immunity in plants. We found that non-RD PRR kinase subfamilies have evolutionary expansion patterns consistent with the NBS-LRR class of PRRs and show lineage-specific expansions marked by tandem duplications. Recently, a similar analysis demonstrated that the RLP class of PRRs (extracellular receptors that lack kinase domains) show these same expansionary patterns [69]. Known PRR kinase subfamilies were three to 12 times larger in rice than in *Arabidopsis*. This finding correlates well with previous studies showing that the rice genome contains over three times as many NBS-LRRs [66]. This is also consistent with the fact that only two IRAK family receptors are known or predicted to confer disease resistance in *Arabidopsis* (FLS2 and PR5K) [17,60], whereas rice and other monocots contain at least five [18,65,59,61, [X. Chen, unpublished data]. All seven of these carry the non-RD motif. Molecular characterization of additional non-RD IRAK receptors will be required to fully comprehend the overall contribution of this class of PRRs to plant innate immunity.

While kinases associated with both plant and animal PRRs carry non-RD motifs, the evolutionary significance of this observation is unclear. Ausubel [70] recently pointed out the need for caution when discussing the origins of similarities between plant and animal innate immune systems [70]. Speculation that these diverse signaling pathways evolved from a common ancestor has often been overstated as the similarities between plant, fly, and human innate immune systems can largely be explained by convergent evolution. With regard to kinases, it is apparent that in some cases, innate immune pathways have lost and/or added specific kinase domains, making such determinations difficult. For example, the *Drosophila* counterpart of human RIP kinase, immune deficiency (Imd), lacks the kinase domain. This pathway is a key component of the *Drosophila* immune system that functions in recognition of gram-negative bacterial pathogens, and thus far no kinases have been identified that associate with the peptidoglycan receptor complex PGRP-LC [16]. Similarly, counterparts of flowering plant NOD-like NBS-LRRs found in the more primitive mosses contain a unique ACF kinase domain that falls within the IRAK family [71]. This kinase-NBS-LRR structure has not been found in flowering plants, suggesting that modern plant NBS-LRRs may have lost the associated kinase. Similarly, the RLP XA21D contains a truncation that results in deletion of the non-RD kinase domain. Despite the loss of the kinase domain, XA21D retains some ability to activate defense responses [20].

The observation that kinases have been lost from or added to PRR-mediated pathways may also underscore the role of these kinases in signaling. Mutagenesis of the kinase domains of RIP1 [72], RIP2 [73], RIP4 [74], IRAK1 [75], and XA21 [76] demonstrate that kinase activity is at least partially dispensable for the innate immune response. This a significant departure from conventional roles of kinases in signaling, and, in the cases of IRAK1 and RIP2, evidence suggests they function as phosphorylation-mediated scaffolding proteins as opposed to signaling through phosphorelay cascades, in which receptor signaling leads to kinase activation followed by phosphorylation relay and signal amplification. Rather, auto- and/or transphosphorylation of IRAK1 [14] and RIP2 [73] promote receptor dissociation and subsequent assembly of signaling complexes, the components of which are not typically IRAK1 or RIP1 phosphorylation substrates. Given that these kinases are highly divergent, it seems likely that these potentially similar signaling mechanisms are the result of convergent evolution. In other instances, such as the *Drosophila* IMD pathway and plant NBS-LRRs and RLPs, assembly of signaling complexes may have evolved to occur in the absence of kinases altogether, hence the lack of these domains [20].

As with other shared features of plant and animal innate immune systems, our findings are also best explained by convergent evolution. Although IRAK, Pelle, and plant receptor kinases are monophyletic (with respect to other kinase families), they fall into distinct clades. Therefore, IRAK1 and Pelle are not more similar to FLS2 or XA21 than they are to plant IRAK family kinases that control nondefense pathways, such as SYMRK or BRI1 (Table S7). In plants, phylogenetic analysis indicates that the PRR subfamilies LRR-XII (FLS2/XA21), SD-2b (PI-2D), and the putative PRR subfamilies LRK10L-2 (LRK10/PR5K) and RLCK-OS2 (similar to RPG1) may have evolved independently to function in innate immunity. These receptor kinases fall into separate clades, have dissimilar extracellular domains, and contain different amino acid substitutions within the RD motif (Tables S4 and S7) [10].

Whether through convergence or divergence, it is unclear why modifications of the RD motif have evolved in kinases that activate innate immune pathways. These changes do not likely represent a single mechanistic adaptation and, thus, may produce distinct mechanistic alterations in each kinase. The prevalence of non-RD kinases in PRRs and PRR complexes does, however, imply that RD kinases may not be ideally suited to control some of the early signaling events that lead to activation of NF-κB in animals and defense responses in plants. This is further supported by the finding that non-RD kinases are also common in mammalian receptor-mediated apoptotic responses that also signal through NF-κB. In mammals, precise control of NF-κB pathways is essential, as persistent activation results in chronic inflammatory syndromes and malignancies [77]. NF-κB signaling and its counterparts in plants and invertebrates may have unique strict regulatory requirements that are not satisfied by RD mechanisms. Alternatively, adaptations to the RD motif may have occurred in response to selection pressures imposed by pathogen effector proteins that aim to disrupt defense signaling. Kinases are known targets of a number of bacterial and viral effectors [78,79]. A reduced role for kinase activity in some non-RD kinases, such as IRAK1, RIP1, RIP2, and XA21, may have evolved to avoid pathogen interference. This may also explain the apparent loss of PRR-

linked kinases in the *Drosophila* IMD and plant NBS-LRR pathways, as well as the presence of some catalytically inactive IRAK family members in humans.

One described commonality between diverse non-RD kinases is a lack of activation loop phosphorylation in some, but not all, non-RD kinases [31]. This is also true for a small number of RD kinases, such as the transforming growth factor-β receptor II kinase domain [80], which maintains a constitutively active configuration through neutralization of the arginine by negatively charged amino acids in the activation loop, and the NFR5 and SYM10 plant IRAK family kinases that lack the activation loop [81]. This function, or lack thereof, has apparently been retained in at least some IRAK family non-RD kinases. In vitro studies have shown that despite the presence of a highly conserved threonine (five amino acids prior to the subdomain VIII motif APE), the activation loop of the rice PRR kinase XA21 is not autophosphorylated [35]. Phosphoproteomics approaches have identified in vivo phosphorylation sites in more than 50 plant receptor kinases [82]. Activation loop phosphorylation was found only in RD and ACF kinases, although non-RD kinases were not well represented in this sample.

Despite indications that the activation loop is not autophosphorylated in some IRAK family non-RD kinases, we found that the highly conserved activation loop threonine is still conserved, indicating that this residue likely plays an important role (unpublished data). Recent data suggest that activation of human IRAK1 [14] and plant IRAK family kinases require this threonine; however, thus far it has only been confirmed as an autophosphorylation site in the plant RD kinases BRI1 [83], BAK1 [83], and SYMRK [84]. As it stands now, these data suggest that this residue may play a critical structural role that is independent of its phosphorylation status. Not surprisingly, some IRAK family kinases appear to utilize alternative regulatory mechanisms. A conserved threonine residue located adjacent to kinase subdomain I is also required for catalytic activity of XA21 [W. Song, personal communication], IRAK1 [14], and SYMRK [84], but not BRI1 [83]. This residue was shown to be phosphorylated in vitro in IRAK1 and XA21 and was identified as an in vivo site in BRI1; however, mutation to alanine only affected the activities of IRAK, XA21, and SYMRK. With the absence of known structures for any IRAK family kinases, structural studies and further phosphorylation site mapping are needed to illuminate the potential functional significance of changes to the RD motif.

Although non-RD kinases do not typically autophosphorylate the activation loop, in some non-RD kinases this regulatory domain can be transphosphorylated by other kinases. In vitro studies have indicated that the activation loop of IRAK1 could be transphosphorylated by the RD kinase IRAK4 [43]. Likewise, the conserved threonine in the activation loop of the non-RD kinase COT was shown to be constitutively transphosphorylated by an unknown kinase in vivo and was required for both robust kinase activity and activation of NF-κB pathways [85]. The authors point out that this is the only known case in which this highly conserved activation loop residue is transphosphorylated. Therefore, in some non-RD kinases transphosphorylation of the activation loop can alter catalytic activity, but whether this occurs in PRR kinases and how it might relate to signaling remain unclear.

The correlation between non-RD kinases in the IRAK and RIP families and innate immunity provides a predictive tool for the functions of unknown IRAK and RIP kinases (currently over 400), the vast majority of which are plant IRAK family kinases. The finding that a kinase is non-RD is clearly not sufficient information by itself to assess function, nor does it preclude a role for non-RD kinases in non-defense pathways, as is the case with *Drosophila* Pelle, which also functions in development [16]. However, it can serve as an important indicator of kinase function. For example, during the course of this study, we were able to predict that the RD kinases IRAK4 [44] and RIP3 [53] would have distinct roles (as compared to IRAK1 and RIP1, respectively) in PRR signaling before their putative functions were published. Our analysis also predicts that the *Caenorhabditis elegans* IRAK family kinase PIK-1 has a likely role in regulating innate immune pathways despite unsuccessful attempts to demonstrate such a role [86]. Similarly, a number of unknown plant IRAK subfamilies can also be predicted to play roles in innate immunity, such as the SD-2a, SD-2b, WAKa, WAKc, RLCK-OS2, and WAKL-OS subfamilies. Indeed, we were informed during the preparation of this manuscript that one of these genes in rice, *Pi-2d,* encodes a receptor in the SD-2b subfamily and confers resistance to the fungal pathogen *Magnaporthe grisea* (X. Chen, unpublished data). The ability to draw associations between enzyme signaling pathway functions and specific conserved functional amino acid residues within broad and diverse enzyme families is a powerful bioinformatics tool.

Materials and Methods

Sequence retrieval and annotation. Amino acid sequences of yeast, fly, worm, and human kinases were obtained from http://www.kinase.com [36,87]. *Arabidopsis* kinases were downloaded from http://hodgkin.mbu.iisc.ernet.in/~king [88]. Divergent kinases that fall within atypical or histidine kinase families were excluded from all analyses. Kinase classifications were done according to Manning et al. [87]. Names of plant Pelle/RLK subfamilies were derived from Shiu et al. [10] with some minor modifications. The WAK group was broken down into three phylogenetically distinct subgroups—WAKa, WAKb, and WAKc—to reflect RD and non-RD classes. We also noted that, although the two human kinases SgK496 and SgK288 have both been referred to as RIP5, SgK496 does not appear to fall within the RIP or IRAK subfamilies [41]. The yeast, fly, worm, human, *Arabidopsis,* and rice kinase domain sequences are available in Datasets S1–S6, respectively.

Characterization of the rice kinome. Sequences encoding kinases in rice were obtained from The Institute for Genomic Research rice database (http://www.tigr.org/tdb/e2k1/osa1). Kinases were identified using two methods. First, reiterative BLAST searches were conducted using representative *Arabidopsis* kinase domains from each kinase family. BLAST results for each hit were manually inspected to obtain significant matches. Subsequently, all putative kinase domains were verified using the kinase domain prediction tool available at KinG (http://hodgkin.mbu.iisc.ernet.in/~king) [88]. This rice kinase sequence set was merged with the PlantsP [89] rice kinome set (M.Gribskov, personal communication) to identify unique kinases. This resulted in 1,508 kinase-encoding genes. Eighty-one genes containing only small kinase fragments were removed for this analysis, and the remaining 1,427 kinases were further analyzed. Kinase family assignments for non-RLKs were obtained from KinG [88].

Identification of non-RD kinases. Multiple alignments for each kinome were done using ClustalW. Alignments were manually adjusted to ensure that conserved kinase motifs were accurately aligned. Each kinase was classified based on the presence or absence of the conserved arginine in kinase subdomain VI and the presence or absence of the conserved lysine in kinase subdomain II, aspartic acid in subdomain VI, and aspartic acid in subdomain VII (K/D/D)

typically required for catalytic activity. Kinases containing the R were classified as RD, and kinases lacking the R were classified as non-RD. Kinases that lacked one or more of the K/D/D residues were classified as ACF. The three subdomains (II, VI, VII) for each RD, non-RD, and ACF kinase are available in Tables S1, S2, and S3. Subfamilies were classified as being predominantly RD, non-RD, or ACF if more than 70% of the kinases fell into one class (Table S6). This was true for all but five subfamilies (RLCK XV, URKI, DUF26-1h, RLCK-OS1, RLCK IXb), which were classified as "mixed." A large number of kinases in rice and *Arabidopsis* were classified as ACF, because these genes encoded protein kinase fragments. While most of these genes in *Arabidopsis* are authentic, many ACF kinases in rice may have been misclassified because of poor annotation resulting from unavailable EST/cDNA sequences.

Statistical analysis. Fisher's exact test was performed using SAS version 8.02 procedure FREQ (SAS Institute, Cary, North Carolina, United States). Fisher's exact test was used instead of Chi-square, since Chi-square analysis does not accurately calculate probabilities with values less than 5. All IRAK family kinases with known or predicted functions were placed into two classes: (1) those with known roles in activating innate immunity or (2) those with other functions. Each kinase was then classified as being RD, non-RD, or ACF. The number of kinases falling into each class was determined to produce a contingency table (Table S7) and a two-tailed probability was calculated. This was compared to the same analysis performed by classifying each kinase according to protein domain structure: (1) receptor kinase containing extracellular LRR domains, (2) receptor kinase containing "other" extracellular domains, or (3) cytoplasmic kinase.

Plant IRAK family genomic distributions. Chromosomal locations of rice kinases were obtained from The Institute for Genomic Research rice database (http://www.tigr.org/tdb/e2k1/osa1). Locations of *Arabidopsis* kinases were obtained from TAIR (http://www.arabidopsis.org). Chromosomal distributions of rice and *Arabidopsis* kinases were visualized using Genome Pixelizer [68]. Each kinase is represented by a colored square; kinases are color-coded according to subfamily. This color-coded image map is included in Figure S1, and an interactive version of the rice distribution map is available at http://rkd.ucdavis.edu.

Supporting Information

Dataset S1. Amino Acid Sequences of All Kinase Domains within the Yeast Kinome

Found at DOI: 10.1371/journal.ppat.0020002.sd001 (38 KB TXT).

Dataset S2. Amino Acid Sequences of All Kinase Domains within the Fly Kinome

Found at DOI: 10.1371/journal.ppat.0020002.sd002 (64 KB TXT).

Dataset S3. Amino Acid Sequences of All Kinase Domains within the Worm Kinome

Found at DOI: 10.1371/journal.ppat.0020002.sd003 (125 KB TXT).

Dataset S4. Amino Acid Sequences of All Kinase Domains within the Human Kinome

Found at DOI: 10.1371/journal.ppat.0020002.sd004 (163 KB EPS).

Dataset S5. Amino Acid Sequences of All Kinase Domains within the *Arabidopsis* Kinome

Found at DOI: 10.1371/journal.ppat.0020002.sd005 (314 KB TXT).

Dataset S6. Amino Acid Sequences of All Kinase Domains within the Rice Kinome

Found at DOI: 10.1371/journal.ppat.0020002.sd006 (1.2 MB EPS).

Figure S1. Chromosome Map Showing the Chromosome Positions for All *Arabidopsis* and Rice Kinases

Kinases are color coded according to subfamily. Images were generated using Genome Pixelizer [68].

Found at DOI: 10.1371/journal.ppat.0020002.sg001 (437 KB PPT).

Table S1. RD Kinases in Yeast, Fly, Worm, Human, *Arabidopsis,* and Rice Kinomes

Motifs for kinase subdomains II, VI, and VII are shown for each kinase.

Found at DOI: 10.1371/journal.ppat.0020002.st001 (1.2 MB XLS).

Table S2. Non-RD Kinases in Yeast, Fly, Worm, Human, *Arabidopsis,* and Rice Kinomes

Motifs for kinase subdomains II, VI, and VII are shown for each kinase.

Found at DOI: 10.1371/journal.ppat.0020002.st002 (451 KB XLS).

Table S3. ACF Kinases in Yeast, Fly, Worm, Human, *Arabidopsis,* and Rice Kinomes

Motifs for kinase subdomains II, VI, and VII are shown for each kinase.

Found at DOI: 10.1371/journal.ppat.0020002.st003 (379 KB XLS).

Table S4. Conservation of the R in Kinase Subdomain VI (RD Motif)

The number of kinases in total and in each kinome containing R or other amino acids are listed. RD motif for all kinases is also shown.

Found at DOI: 10.1371/journal.ppat.0020002.st004 (642 KB XLS).

Table S5. Cross-Kingdom Conservation of Non-RD Kinases (Complete Listing)

All non-RD kinases and their counterparts in other kinomes are shown. Kinases are separated into color-coded groups. ACF and RD kinases are indicated when present as homologs of non-RD kinases. Kinomes that lack homologs have been left blank.

Found at DOI: 10.1371/journal.ppat.0020002.st005 (32 KB XLS).

Table S6. Classification of Plant IRAK Subfamilies

Numbers of rice and *Arabidopsis* RD, non-RD, and ACF kinases are listed for each subfamily. Subfamilies that have recently expanded in rice and/or *Arabidopsis* are listed separately. Chromosomes containing clusters of four or more closely linked kinases are indicated.

Found at DOI: 10.1371/journal.ppat.0020002.st006 (45 KB XLS).

Table S7. Raw Data Used for Statistical Analysis of IRAK Family Kinase Functions and Non-RD Motifs

The contingency table classes (RD, Non-RD, ACF) or (LRR RK, Other RK, Cytoplasmic) and putative function for each kinase are indicated. The resulting 2×2 contingency table is shown. Also, a cladogram depicting phylogenetic relationships between known IRAK family members is shown. The tree was constructed using ClustalW and visualized with Phylodraw v.0.8 (Graphics Application Lab, Pusan National University, Pusan, South Korea). Bootstrap values (100 trials) are written on branch nodes. PRR kinases are indicated with red lines and are found within at least three distinct clades.

Found at DOI: 10.1371/journal.ppat.0020002.st007 (60 KB XLS).

Table S8. Chromosome Numbers and Positions of 5′ End for Each *Arabidopsis* Kinase

Plant IRAK family kinases are highlighted in yellow.

Found at DOI: 10.1371/journal.ppat.0020002.st008 (1.7 MB XLS).

Table S9. Chromosome Numbers and Positions of 5′ End for Each Rice Kinase

Plant IRAK family kinases are highlighted in yellow.

Found at DOI: 10.1371/journal.ppat.0020002.st009 (884 KB XLS).

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