REVIEW SUMMARY

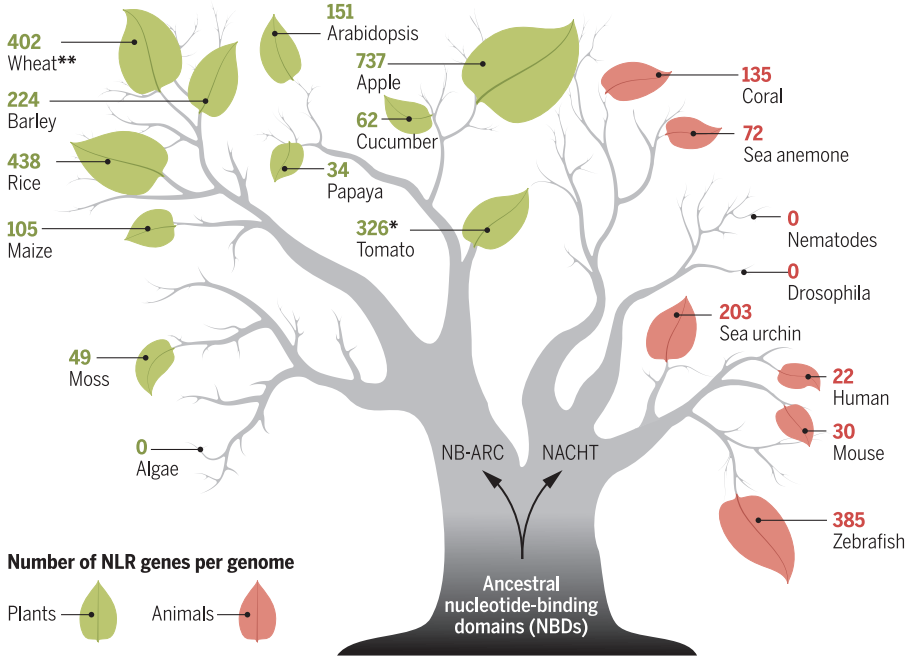
INNATE IMMUNITY

Intracellular innate immune surveillance devices in plants and animals

Jonathan D. G. Jones,*† Russell E. Vance,*† Jeffery L. Dangl*†

BACKGROUND: Pathogens cause agricultural devastation and huge economic losses. Up to 30% of our crops are lost before or after harvest to pathogens and pests, wasting water and human effort. Diseases and pests are major problems for sustainable agriculture in the face of population growth. Similarly, microbial infection remains a major cause of human mortality and morbidity, responsible for ~25% of deaths worldwide in 2012. We lack vaccines for several major infectious diseases, and antibiotic resistance is an ever-growing concern.

Plant and animal innate immune systems respond to pathogen infection and regulate beneficial interactions with commensal and symbiotic microbes. Plants and animals use intracellular proteins of the nucleotide binding domain (NBD), leucine-rich repeat (NLR) superfamily to detect many kinds of pathogens. Plant and animal NLRs evolved from distinct derivatives of a common ancestral prokaryotic adenosine triphosphatase (ATPase): the NBD shared by APAF-1, plant NLR proteins, and CED-4 (NB-ARC) domain class and that shared by apoptosis inhibitory protein (NAIP), CIITA, HET-E, TP1 (NACHT) domain class, respectively. Animals and fungi can carry both NB-ARC and NACHT domain proteins, but NACHT domain proteins are absent from plants and several animal taxa, such as *Drosophila* and nematodes. Despite the vast evolutionary distance between plants and animals, we describe trans-kingdom principles of NLR activation. We propose that NLRs evolved for pathogen-sensing in diverse organisms because the flexible protein domain architecture surrounding the NB-ARC and NACHT domains facilitates evolution of "hair trigger" switches, into which a virtually limitless number of microbial detection platforms can be integrated.



NLR tree. Evolution of NLR genes followed diverging pathways for plant and animal species. Numbers of NLR genes per genome identified computationally range widely, as shown on this stylized evolutionary tree (branches not to scale). The numbers of NLRs can vary markedly even across genomes from closely related taxa. NLRs likely derived from a common ancestor that expressed both NACHT and NB-ARC type NBDs. NACHT is found in animal NLRs, and NB-ARC in plant NLRs. Both occur in fungi. A variety of N- and C-terminal domains have been evolutionarily recruited onto NBDs, including those characteristic of NLRs. The asterisk for tomato indicates that experimental evidence exists to give this precision, as discussed in the main text. The double asterisk for wheat indicates the number of NLRs per diploid genome (wheat is hexaploid). NLR-like fungal proteins lack the LRR domain characteristic of NLRs and are thus not included here.

ADVANCES: Structural biology is beginning to shed light on pre- and postactivation NLR architectures. Various detection and activation platforms have evolved in both plant and animal NLR surveillance systems. This spectrum ranges from direct NLR activation, through binding of microbial ligands, to indirect NLR activation after the modification of host cellular targets, or decoys of those targets, by microbial virulence factors. Homo- and heterotypic dimerization and oligomerization of NLRs add complexity to signaling responses and can enable signal amplification. NLR population genomics across the plant and animal kingdoms is increasing owing to application of new capture-based sequencing methods. A more complete catalog of NLR repertoires within and across species will provide an enhanced toolbox for exploiting NLRs to develop therapeutic interventions.

ON OUR WEBSITE

Read the full article at http://dx.doi.org/10.1126/science.aaf6395

OUTLOOK: Despite breakthroughs in our molecular understanding of NLR activation, many important questions remain. Biochemical mechanisms of NLR activation remain obscure. Events downstream of plant NLR activation and outputs such as transcription of defense genes, changes in cell permeability, localized cell death, and systemic signaling remain opaque. We do not know whether activated plant NLRs oligomerize or, if they do, how this is achieved, given the diversity of subcellular sites of activation observed for various NLRs. It is not clear whether and how the different N-terminal domains of plant NLRs signal. We have increasing knowledge regarding how animal NLRs assemble and signal, although knowledge gaps remain. Therapeutic interventions in humans targeting NLRs remain on the horizon. Design of novel recognition capabilities and engineering of new or extended NLR functions to counter disease in animals and plants provides tantalizing future goals to address plant and animal health problems worldwide. ■

The list of author affiliations is available in the full article online.
*These authors contributed equally to this work.
†Corresponding author. Email: jonathan.jones@sainsbury-laboratory.ac.uk (J.D.G.J.); rvance@berkeley.edu (R.E.V.); dangl@email.unc.edu (J.L.D.)
Cite this article as J. D. G. Jones *et al.*, *Science* **354**, aaf6395 (2016). DOI: 10.1126/science.aaf6395

REVIEW

INNATE IMMUNITY

Intracellular innate immune surveillance devices in plants and animals

Jonathan D. G. Jones,[1]*† Russell E. Vance,[2]*† Jeffery L. Dangl[3]*†

Multicellular eukaryotes coevolve with microbial pathogens, which exert strong selective pressure on the immune systems of their hosts. Plants and animals use intracellular proteins of the nucleotide-binding domain, leucine-rich repeat (NLR) superfamily to detect many types of microbial pathogens. The NLR domain architecture likely evolved independently and convergently in each kingdom, and the molecular mechanisms of pathogen detection by plant and animal NLRs have long been considered to be distinct. However, microbial recognition mechanisms overlap, and it is now possible to discern important key trans-kingdom principles of NLR-dependent immune function. Here, we attempt to articulate these principles. We propose that the NLR architecture has evolved for pathogen-sensing in diverse organisms because of its utility as a tightly folded "hair trigger" device into which a virtually limitless number of microbial detection platforms can be integrated. Recent findings suggest means to rationally design novel recognition capabilities to counter disease.

Multicellularity creates nutrient niches for microbial colonization, which in turn drives natural selection for hosts with effective innate immunity. In plants and animals, innate immunity involves both cell surface receptors (*1*) and intracellular receptors of the NLR [nucleotide binding domain (NBD) and leucine-rich repeat (LRR)] superfamily (*2*–*4*). NLRs play critical roles in organismal health in both plants and animals; absence or dysfunction can result in organ failure and death (Fig. 1). NLRs were originally referred to as "Nod-like receptors," but we do not favor this name because it arose only in the mammalian literature, and the official consensus nomenclature is that NLR stands for "NBD-LRR domain-containing" (*5*). Plant NLRs are present in angiosperms and gymnosperms, and even in bryophytes and liverworts, but not in the single-celled alga *Chlamydomonas*. NLR immune receptors are also found in diverse animals, from corals, sea urchins (*6*, *7*), and primitive chordates (*8*) to fish (*9*) and mammals (*10*). Even though chordates and plant genomes carry NLR genes, genes encoding these proteins have not been found in several animal lineages such as nematodes and arthropods. Yet, NLR-like proteins with similar core architecture, but lacking LRR domains, are present in filamentous fungi, where they can play a role in heterokaryon incompatibility (*11*, *12*). Thus, NLRs and fungal NLR-like proteins represent a protein architecture deployed across kingdoms for host defense and/or self-nonself discrimination. In this Review, we focus on an illustrative handful of the most exciting current conceptual developments in NLR biology and refer the reader to excellent recent publications for further details (*13*–*19*). We hope to convey the enthusiasm of this rapidly advancing field as an area of active basic research that is at the cusp of exploitation to address pressing plant and animal health problems worldwide.

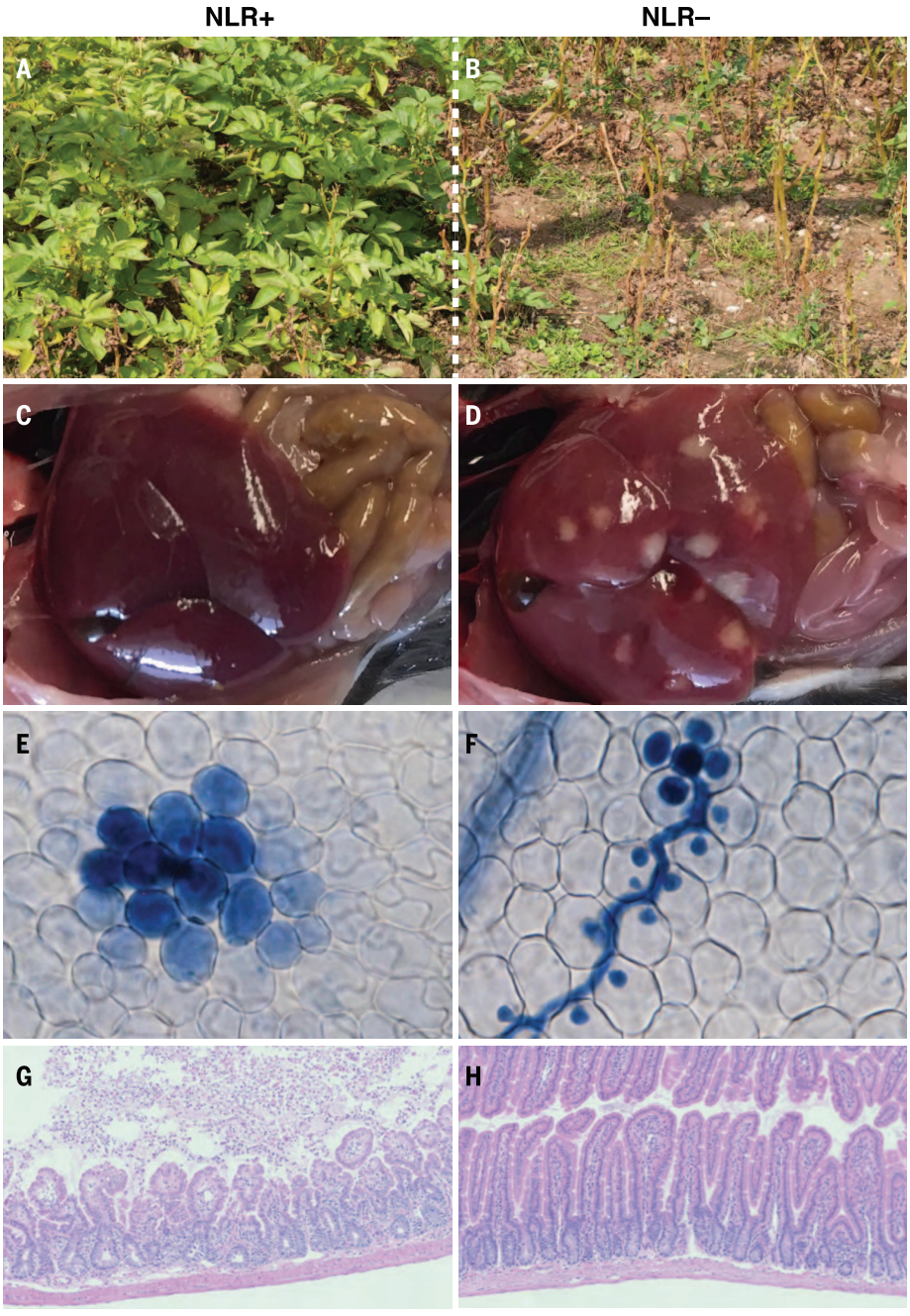
[1]Sainsbury Lab, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK. [2]Howard Hughes Medical Institute (HHMI), Department of Molecular and Cell Biology, and Cancer Research Laboratory, University of California, Berkeley, CA 94720, USA. [3]Howard Hughes Medical Institute, Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280, USA.
*These authors contributed equally to this work. †Corresponding author. Email: jonathan.jones@sainsbury-laboratory.ac.uk (J.D.G.J.); rvance@berkeley.edu (R.E.V.); dangl@email.unc.edu (J.L.D.)

NLR architecture: Evolution sculpts sensitive switches

Plant and animal NLRs share a similar modular domain architecture, including the core NBD and LRR domain, although in both clades there is substantial diversity in N- and C-terminal accessory domains (Fig. 2). The NBD falls within the STAND [signal transduction adenosine triphosphatase (ATPases) with numerous domains] AAA^+ ATPase superfamily, which typically includes Walker A (P-loop) and Walker B motifs involved in nucleotide binding and hydrolysis (*20*, *21*). The NBD is associated with adjacent α-helical domains (*22*–*24*). Plant NLRs use a subtype of STAND NBD called the NB-ARC (nucleotide-binding, Apaf1, Resistance, CED4), associated with two α-helical domains. This domain, also known as the Apoptotic ATPase (Ap-ATPase) domain (*25*), is shared with animal proteins that lack LRRs and are involved in apoptosis such as mammalian Apaf-1, *Drosophila* DARK and nematode CED4, and likely evolved from a class of prokaryotic ATPases. Animal NLRs, in contrast, carry a distinct NBD subtype, the NACHT (NAIP, CIITA, HET-E, and TP1) domain, associated with three α-helical domains, that also likely derived from a distinct prokaryotic ancestral domain (*20*, *22*, *26*). Both NACHT and NB-ARC domains are in fungi, where they have recruited diverse N- and C-terminal domains but not LRRs (*12*). Thus, plant and animal NLRs likely evolved from distinct ancestral NBD lineages based on differential expansion from a common ancestor of these STAND AAA^+ ATPases (*27*).

Although the focus of this Review is on NLRs involved in innate pathogen-detection, some mammalian NLRs appear to have distinct functions, including transcriptional regulation in adaptive immunity (*28*, *29*). Nevertheless, despite considerable NLR diversity in sequence and function, all NLR and NLR-like proteins are presumed to involve a similar switch-like activation mechanism. Indeed, studies of Apaf1 and its homologs have established the paradigm for our current understanding of NLR activation (*30*). In this model, preactivation states of NLR proteins feature intra- and potentially intermolecular domain interactions to keep the NBD conformational equilibrium in a suppressed but not fully inactive state (*17*, *31*, *32*). In response to specific pathogen effector (virulence) proteins or other specific stimuli, the intramolecular interactions are altered, and the NBD is believed to exchange adenosine diphosphate (ADP) for adenosine triphosphate (ATP), likely driving NLR oligomerization in at least some cases. NLRs can hydrolyze ATP to ADP, but this activity does not drive oligomerization. Whether ATP hydrolysis plays an important role in NLR regulation is unclear; ATP hydrolysis may convert activated NLRs to an inactive state.

NLR oligomerization is believed to initiate signaling by the proximity-induced recruitment and activation of downstream molecules via N-terminal accessory signaling domains (*33*). These N-terminal domains vary considerably (Fig. 2) but are commonly coiled-coil or TIR domains in plant NLRs, or domains in the death-fold superfamily (such as CARD or Pyrin domains) in animal NLRs. The putative signaling molecules recruited to plant NLRs have not been identified, but several such molecules recruited to animal NLRs have been described. These include a kinase (RIPK2) that is recruited to NOD1/2, a protease (caspase-1) that is recruited directly to NLRC4 and NLRP1, and a Pyrin-CARD–containing adaptor protein (ASC) that recruits caspase-1 indirectly to several NLRs, including NLRP3. Interestingly, the pyrin domain of NLRP3 is also believed to propagate signaling by nucleating the oligomerization of ASC into polymerized filaments that coalesce into massive intracellular "specks" (*34*). The essentially irreversible conversion of ASC from a soluble to filamentous form is reminscent of the biochemical behavior of prions, and indeed, the ASC Pyrin domain exhibits prion-like properties when expressed in yeast. Mutations in ASC that disrupt its prion activities in yeast also abrogate its ability to signal in mammalian cells (*35*). Conversely, a yeast prion domain can functionally replace the N-terminal Pyrin domain in ASC (*35*). Highly cooperative polymerization that produces a "hair-trigger" all-or-none signaling output might be



CREDITS: (A) AND (B), KAMIL WITEK/(C) AND (D), VIVIEN MALTEZ AND ED MIAO/(E) AND (F), JANE PARKER/(G) AND (H), ISABELLA RAUCH

Fig. 1. NLRs make a major contribution to organismal health. (**A** and **B**) Isogenic potato plants that either (A) express or (B) lack a specific NLR (*Rpi-vnt1*) conditioning resistance to the oomycete pathogen *Phytophthora infestans*, the causal agent of potato late blight. (**C** and **D**) Livers from (C) normal NLRC4^{+} (resistant) and (D) NLRC4-deficient (susceptible) mice after lethal infection with *Chromobacterium violaceum* (white bacterial lesions are visible). (**E**) *Arabidopsis* expressing the NLR-encoding *RPP5* gene undergoes protective localized cell death (trypan blue–stained cells; debris of dead pathogen stained intensely in the center) in response to the *Arabidopsis* downy mildew pathogen (*Hyaloperonospora arabidopsidis*) strain NoCo2. (**F**) Successful downy mildew colonization of leaf tissue from isogenic susceptible *Arabidopsis* that lack *RPP*5 (trypan blue–stained hyphae and intracellular pathogen haustoria). (**G**) Destruction of mouse intestinal tissue after NLRC4 activation showing sloughing of epithelial cells into the intestinal lumen. The sloughing response is believed to prevent bacterial invasion into deeper tissue. (**H**) Normal mouse intestinal tissue showing elongated villi and intact intestinal epithelium.

desirable in proteins that need to respond rapidly and sensitively to pathogen invasion. It is tempting to speculate that polymerization may be a common feature of signaling downstream of both plant and animal NLRs, although this remains to be established experimentally.

In both plant and animal NLRs, deletion of the LRR domain can result in constitutive NLR activation. Thus, a primary function of the LRR domain is likely to be negative regulation of NBD-mediated oligomerization. A structure of mouse NLRC4 suggests that autoinhibition is at least partly mediated by direct contact between the NBD and LRR (*36*), although whether this is generalizable to all NLRs remains to be seen. Once activated, NLRs often induce a characteristic cell death response termed the "hypersensitive response" in plants and "pyroptosis" in animals. Cell death is believed to restrict pathogen replication at the site of infection and, in animals, results in the release of soluble mediators that recruit and activate additional immune cells. Inappropriate spontaneous NLR activation can lead to autoimmune conditions in both plants and animals. These syndromes can be more severe at cold temperatures in both plants and animals, resulting in chilling sensitive ectopic cell death in plants and familial cold autoinflammatory syndrome (FCAS) in humans (*37*, *38*). These examples suggest that intra- or intermolecular NLR interactions required for autoinhibition can be perturbed at the nonpermissive temperature, or that ATP hydrolysis is attenuated at lower temperatures, shifting the equilibrium from the inactive toward the activated state.

Although the induced oligomerization model has underpinned investigations of animal NLR activation, it has not been demonstrated for plant NLRs. Activation of the tobacco N and *Arabidopsis* RPP1 NLRs correlates with self-association (*39*, *40*), but whether this represents the formation of oligomers, as in the case of Apaf1 and some animal NLRs, or simply homodimers remains unclear. There may be diversity in both the resting-state architectures of plant NLRs and in their modes of activation by microbial signals that are tuned by natural selection; no universal generalization may exist. For example, heterogeneity in the subcellular site of activation of a given NLR is potentially determined by the precise localization of the microbial effector target that activates it. There may be additional structural constraints imposed by requirements for interaction with partner "helper" NLRs, with microbial effector target domains, or decoys of these, or because of integration of target decoy domains into the NLR itself (*3*, *41*–*44*).

Subsequent to NLR activation, plant and animal innate immunity mechanisms likely differ greatly, although we remain quite ignorant of postactivation mechanisms in plants. Nevertheless, despite remarkable diversity in upstream and downstream signaling events, we are forced to contemplate what is so fundamentally advantageous about the NLR architecture that could explain why it arose convergently in plants and animals to play a role in pathogen detection and

defense activation (27, 45). The full spectrum of mechanisms in each kingdom suggests that there is scope for more conceptual similarities than previously suspected. Given this diversity, we propose that one advantage of the NLR architecture may simply be its ability to function as a robust on-off switch in diverse signaling contexts. To illustrate the flexibility of the NLR architecture, we articulate below four distinct mechanisms of pathogen sensing ("direct," "guard," "decoy," and "integrated decoy") (Fig. 3) and discuss how these four mechanisms can be applied to individual and paired plant and animal NLRs. It remains to be seen whether these four mechanisms are the main modes of action of all NLRs or whether we are only scratching the surface.

Division of labor: Sensors and helpers

Specific NLR responses can require a pair of NLR proteins in which one senses the ligand whereas the other (the "helper NLR") is required for its downstream signaling (46, 47). In mammals, the NAIP/NLRC4 inflammasomes are composed of sensor/helper NLR pairs. In this system, a NAIP sensor NLR is activated by direct binding to a specific bacterial protein ligand (such as flagellin) (48, 49), leading to recruitment of NLRC4 as a helper NLR. NLRC4 does not appear to bind directly to ligands but instead functions downstream of NAIPs to recruit and activate caspase-1, a key executioner of inflammasome signaling pathways. Structural analyses demonstrate that in the absence of stimulation, NLRC4 (and presumably NAIPs) are retained in the cytosol as monomers, autoinhibited via intradomain interactions (36, 50, 51). Recognition of microbial molecules by NAIPs exposes a donor "catalytic" surface on the NAIP that binds to a "receptor" surface on NLRC4, provoking a conformational change in NLRC4 that exposes its catalytic surface. This in turn propagates the recruitment of additional NLRC4 monomers to form a ring-shaped oligomer that appears to contain one NAIP and 9 to 11 NLRC4 molecules. This striking stoichiometry suggests that NAIP activation is a hair trigger for NLRC4-mediated signal amplification.

The concept of sensors and helpers also applies to some plant NLRs. Plant genomes encode variable numbers of NLRs, with an atypical N-terminal coiled-coil domain called a CC-R (52) that correlates with helper NLR function. Resistance to the Tobacco Mosaic Virus requires both the sensor TIR NLR protein N and the helper CC-R NLR protein NRG1 (53). In *Arabidopsis*, the five CC-R–encoding genes comprise two paralogous NLR families that function as helper NLRs (47, 54). One of them, a member of the ADR CC-R family, has both canonical, P-loop–dependent signaling functions in cell death control and a noncanonical P-loop–independent function as a helper NLR for several effector sensor NLRs. The noncanonical function suggested a requirement as a scaffold, much like NLRC4, which can also exhibit P-loop–independent functions in transduction of effector-activated NAIP signals (48). A given NLR might be operating by both of these mechanisms, depending on the activation context, as demonstrated for ADR1-L2 (54). Little is known about how the CC-R domain is integrated into activation mechanisms, but the fact that it is evolutionarily ancient and monophyletic suggests a generalizable function in plant NLR biology.

Natural NLR variants featuring degenerate NBD consensus sequences exist, and there is evidence to suggest that they may participate in noncanonical activation mechanisms. The rice Pb1 NLR family lacks a P-loop motif but nonetheless conditions broad spectrum resistance to rice blast, potentially by acting as a helper NLR (55). This is likely evolutionarily conserved, because there are Pb1 homologs in maize. The small collection of *Arabidopsis* and *Arabidopsis lyrata* proteins containing variant P-loop residues is also likely to alter or impair the canonical activation mechanism outlined above (56). These include NLRs with integrated decoy domains that function in NLR pairs that are encoded together and function together. For example, in the RPS4/RRS1 gene pair, RRS1 is the sensor NLR, and its P-loop is not required to activate signaling (57).

Guards and decoys: Getting the most from the NLR receptor repertoire

Plant NLRs were first revealed by cloning *Resistance* (*R*) genes that confer the capacity to activate defense upon detection of specific pathogen effectors. There is selective pressure for pathogens to mutate their effectors to evade NLR-dependent surveillance, which in turn selects for evolution of either new *R* gene alleles, or other *R* genes, that restore resistance. This "gene-for-gene" coevolution led to the hypothesis that NLR proteins might directly interact with the recognized effector, and in some cases this prediction was fulfilled (58). However, in most cases, direct interaction between a plant NLR and the "recognized" effector is not observed. Instead, many plant NLRs appear to monitor the state of self proteins, termed "guardees," whose primary function is in defense signaling and as such are frequently targeted by pathogen effectors. If a pathogen virulence protein alters the guardee's structure, then the associated NLR is activated. The "guard" strategy thus allows a relatively small repertoire of NLRs (~150 in *Arabidopsis*) to protect against diverse pathogen effectors (3, 59, 60). For example, NLR proteins RPM1 and RPS2 act at the plasma membrane to monitor the state of the plasma membrane–associated defense regulator RIN4. RPM1 detects phosphorylation of a specific threonine residue on RIN4 provoked by the pathogen effectors AvrB or AvrRpm1 (61, 62) and mediated via a receptor-like cytoplasmic kinase (63). This effector-modulated phosphorylation interferes with both RIN4-dependent mesophyll defense responses (62) and stomatal immunity (64). In contrast, RPS2 is activated by cleavage of RIN4 by the bacterial cysteine protease effector AvrRpt2 (65, 66).

Similarly, RPS5 monitors the state of protein kinase PBS1, also at the plasma membrane (67, 68). PBS1 is targeted for proteolytic cleavage by plasma membrane–localized AvrPphB. Illustrating the

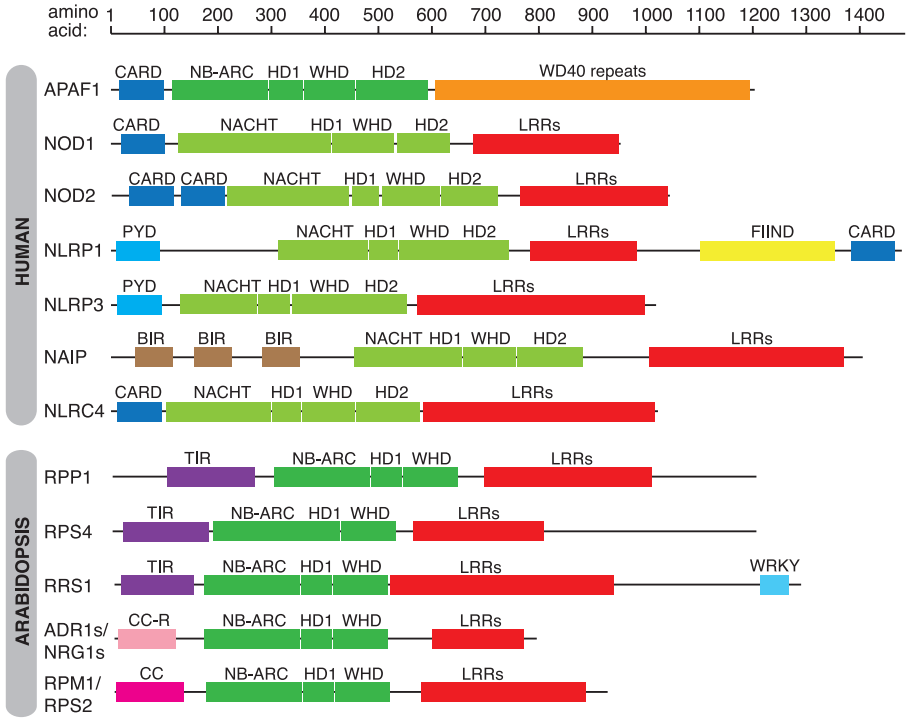


Fig. 2. Diversity of NLR and NLR-like architectures ("NLR-o-gram"). Domain structures of representative well-studied NLR proteins from human and *Arabidopsis* are shown approximately to scale. Definitions of acronyms are provided in Box 1. The NACHT and NB-ARC domains are sometimes defined as including the associated helical domains, but these domains are shown separately here for clarity. Humans contain additional NLRs not known to be directly involved in pathogen sensing.

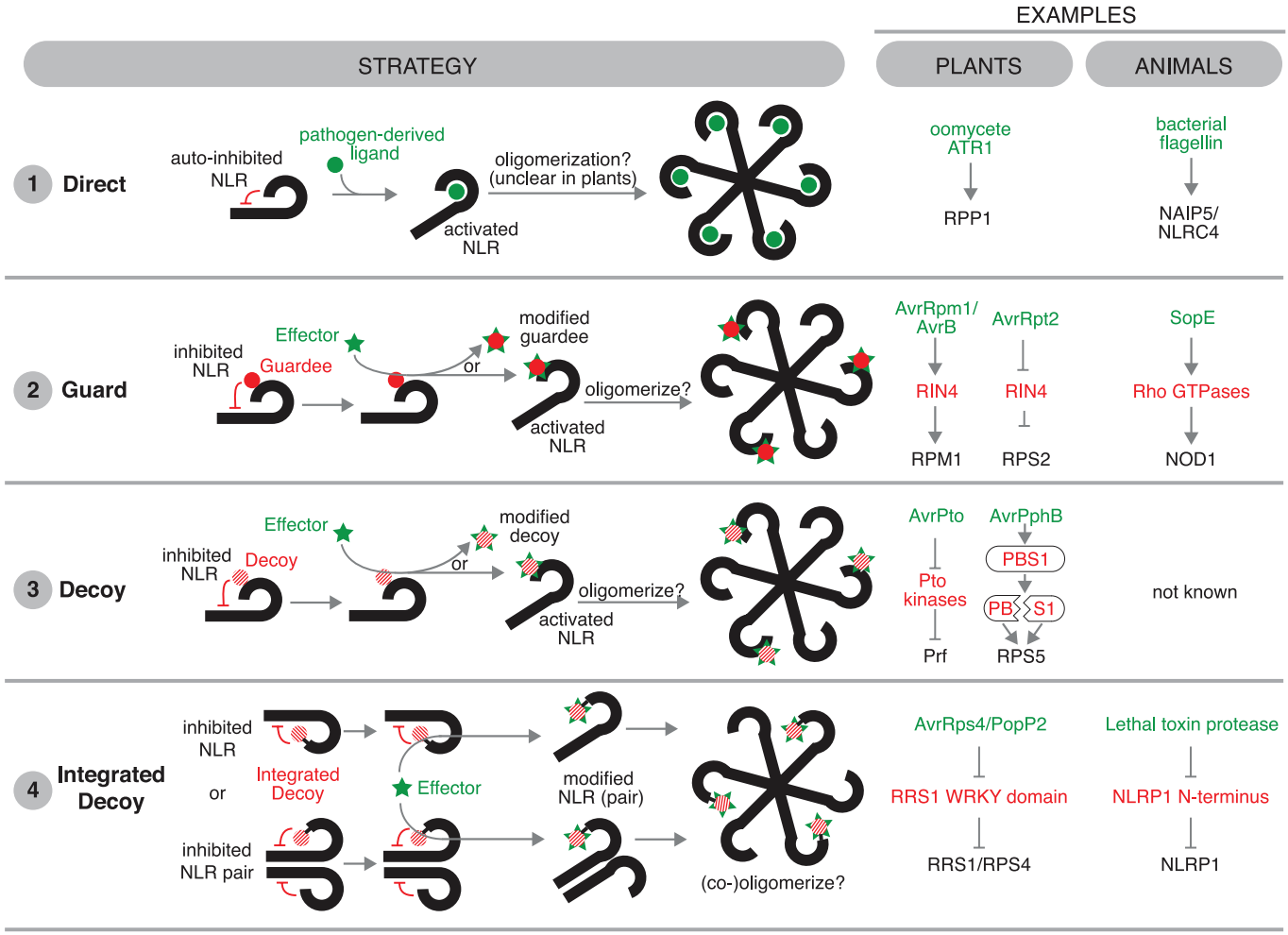


Fig. 3. Diverse strategies for NLR-mediated detection of pathogens. Four conceptually distinct strategies are illustrated. The details of how each strategy is implemented for a specific NLR example may vary. The guard and decoy strategies are analogous: In both cases, the guardee or decoy proteins are involved in maintaining the NLR in an inhibited state, and in both cases, the inhibition is relieved upon effector-mediated modification of the guardee or decoy. Guardees are distinguished from decoys by having an additional and separate function in host defense, whereas decoys are merely mimics of host defense proteins. Guardees are thus the "intended" targets of effectors, whereas decoys are inadvertently targeted by effectors.

flexibility of the guard strategy, the RPS5/PBS1 system has been engineered to expand RPS5 function to recognize other protease cleavage sites engineered into PBS1 (*69*).

Mutations of a guarded protein, such as PBS1, can result in no enhanced disease susceptibility. This suggests that paralogs of genuine targets of virulence proteins may have evolved to resemble that target, thus "luring" pathogens to reveal themselves by their action on a protein that is not required for defense. This concept was elaborated as the "decoy" model (*41*). Given the extensive functional redundancy of plant proteins, it is formally difficult to distinguish between redundant guardees and decoys; absence of evidence that a given protein is involved in host defense is not sufficient evidence to prove that it is a decoy. Nevertheless, PBS1 is likely a decoy for a class of cytosolic kinases that function during defense activated by cell surface receptors (*63*, *70*). Additionally, tomato NLR Prf has evolved an extended N-terminal domain of unknown function that acts as a detection platform with which to monitor effector-targeted protein kinases of the Pto family that are, in turn, likely decoys for the defense-relevant kinase domains of pattern recognition receptor LRR-kinases (*71*). Likewise, the *Arabidopsis* NLR ZAR1 monitors the state of multiple pseudokinases (*72*) that are likely decoys for effectors that target receptor-like cytoplasmic kinases involved in defense signaling.

In mammals, the concepts of guards and decoys have not been extensively discussed. The mammalian NOD1 and NOD2 NLRs were originally proposed to function as direct detectors of bacterial peptidoglycan fragments, which is consistent with a direct ligand-receptor model for NLR activation in animals (*73*–*75*). However, crystallographic proof that NOD1 and NOD2 bind directly to peptidoglycan-derived ligands is lacking. Instead, NOD1 and NOD2 might indirectly sense pathogens via responsiveness to disruption of host cell physiology (*76*, *77*). For example, activation of the host cytoskeletal regulators Rac1 and Cdc42 by the secreted *Salmonella* virulence factor SopE results in NOD1 activation (*76*). NOD1 and NOD2 also appear to be responsive to pathogen-induced ER stress (*77*). These results are consistent with NOD1 and NOD2 exhibiting guard-type activation, although it remains unclear how this is to be reconciled with genetic evidence that NOD1 and NOD2 can also be activated by peptidoglycan.

Mammalian NLRP3 also acts as a guard of cellular integrity because it can be activated by disruption of cellular ion gradients. The molecular mechanism of NLRP3 activation remains unresolved, but the essential role of NEK7 kinase in NLRP3 activation (*78*–*80*) is reminiscent of the role of protein kinases in plant NLR activation. Thus, the guard-type activation mechanism first described in plants may also be germane to NLR-mediated pathogen detection in animals.

Integrated decoys

An evolutionary challenge for sensor/helper, guard/guardee, or guard/decoy NLR systems is that if the corresponding *NLR* genes are unlinked and exhibit allelic variation, inappropriate allelic

Box 1. Definitions for the acronyms used in this paper.

ADR1: Activated disease resistance 1
ASC: Apoptosis-associated speck-like protein containing a CARD
BIR: Baculovirus inhibitor-of-apoptosis repeat
CARD: Caspase activation and recruitment domain
CC-R: Coiled coil domain-RPW8-like
CC: Coiled coil
FIIND: Function-to-find domain
HD1/2: Helical domain 1/helical domain 2
HMA: Heavy metal associated (same domain as RATX)
LRRs: Leucine-rich repeats
NACHT: NAIP, CIITA, HET-E, TP1 domain
NAIP: NLR family, apoptosis inhibitory protein
NB-ARC: Nucleotide binding domain shared by APAF1, R genes, CED-4
NBD: Nucleotide binding domain
NLR: Nucleotide binding domain, leucine-rich repeat–containing
NLRC4: NLR family, CARD domain–containing 4
NLRP1/3: NLR family, Pyrin domain–containing 1 and 3
NOD1/2: Nucleotide-binding oligomerization domain–containing 1 and 2
PBS1: AvrPphB susceptible 1
PYD: Pyrin domain
RIPK2: Receptor interacting protein serine/threoning kinase 2
RATX: Related to ATX1 (same domain as HMA)
RGA5: Resistance gene analog5
RIN4: RPM1 interacting protein 4
RPM1: Resistance to Pseudomonas maculicola 1
RPP1: Resistance to Peronospora parasitica 1
RPS4/5: Resistance to Pseudomonas 4 and 5
RRS1: Resistance to Ralstonia solanacearum 1
STAND: Signal transduction ATPases with numerous domains
TIR: Toll-like, interleukin-1 receptor resistance protein
WD40: tryptophan (W), aspartate (D) 40 amino acids
WHD: Winged Helix Domain
WRKY: tryptophan (W), arginine (R), lysine (K), tyrosine (Y) motif-containing domain

combinations may be generated that can result in microbe-independent autoimmunity (*81*, *82*). Such untoward consequences may facilitate selection for genetic linkage of *NLR* pairs that function together. Intriguingly, in multiple plant genomes a given *NLR* may be closely linked and divergently transcribed from another *NLR* gene that is required for its function. For example, rice RGA4 and RGA5 are two linked NLRs required for recognition of two effectors from the rice blast pathogen (*Magnaporthe oryzae*) (*83*). Similarly, *Arabidopsis* RPS4 and RRS1 are two linked NLRs that are both required to confer recognition of two bacterial effectors, AvrRps4 and the YopJ family acetyltransferase PopP2, and an unidentified fungal molecule (*84*). In both of these examples, the two NLR components appear to be preassociated (*57*) rather than associating only upon effector perception, as occurs with NAIPs and NLRC4 (*48*).

RRS1 carries a WRKY transcription factor (TF) DNA binding domain toward its C terminus. *Arabidopsis* encodes ~90 WRKY TFs, many of which are implicated in innate immunity in plants (*85*). Conceivably, then, WRKY proteins might be targets for pathogen effectors because their inactivation should result in elevated susceptibility. Recent data (*86*, *87*) fulfill this expectation: The effector PopP2 acetylates two lysines in the canonical WRKYGQK DNA-binding motif, and the GQK lysine is crucial for RPS4/RRS1–dependent defense activation. Another unrelated bacterial effector, AvrRps4, also binds to the WRKY domain. The RPS4/RRS1 complex converts the effector-dependent modification of the RRS1 WRKY domain into defense activation. Integration of the guarded decoy domain into RRS1, an indispensable and linked partner to RPS4, reduces the risk of recombination giving rise to inappropriate allelic combinations of what could be three unlinked proteins, which might result in autoimmunity. There are at least nine such linked NLR pairs in the reference *Arabidopsis* genome. Importantly, these exhibit decoy domain diversity at orthologous positions across the Brassicaceae, suggesting that the rapid shuffling of integrated decoy domains onto existing functional *NLR* pairs is a useful evolutionary strategy.

The discovery of linked paired NLRs in plants creates a new opportunity. Functional transfer of plant NLRs across species barriers has proven largely impossible. This restricted taxonomic functionality is poorly understood but, if solved, could greatly enhance prospects for crop disease control through genetics rather than chemistry. For example, RPS4 and RRS1 confer effector recognition and *Colletotrichum* fungus resistance when cotransformed into Solanaceae or Cucurbitaceae (*88*). This is consistent with the idea that restricted taxonomic functionality for one NLR arises from a requirement for the appropriate helper or partner NLR. The additional required NLRs are usually hard to identify, but in the case of paired NLR genes, comprise each other. Systematic transfer of paired NLRs between plant taxa may provide additional recognition capacities that would enable elevated crop disease resistances.

Overcoming taxonomic functionality restriction may require more than paired NLR genes. The tomato Prf/Pto guard/guardee pair confers recognition of two widespread *Pseudomonas* effectors but does not appear to function outside the Solanaceae. Prf/Pto function requires the helper NLRs NRC2a, 2b, and NRC3 (*46*). A fuller understanding of how sensors functionally integrate with helpers is required to rationally expand and transfer useful disease resistance.

The concept of integrated decoys may be widely applicable (*42*). Genome-wide analyses of plant NLR genes led to the discovery of many integrated domains in plant NLR proteins (*42*–*44*). There is an overlap between the list of integrated domains and the list of domains found to be frequent interactors of pathogen effectors in large-scale yeast 2-hybrid screens (*43*, *89*). This correlation is consistent with the view that selection favors integration into NLRs of protein domains that are targets, or decoys of targets, of pathogen effectors. For example, the rice NLR RGA5 carries a C-terminal RATX1 (related to yeast copper transporter ATX1) or HMA (heavy metal–associated) domain and likely binds metals. Effectors AVR-Pia and AVR1-CO39 from *M. oryzae* interact with this domain and trigger RGA4-dependent defense. Another rice gene pair, Pikp-1 and Pikp-2, recognizes a different effector, Avr-Pik, which exists in the fungal population as a series of alleles (AvrPikA-D). Structural studies have illustrated how effectors interact with the HMA domain that is located between the CC and the NB-ARC domain of Pikp-1 (*90*). But why do pathogens evolve effectors that interact with HMA domain proteins? A clue is provided by the observation that the recessive disease-resistance gene *Pi21* contains an HMA domain (*91*). Recessive disease resistance genes are typically interpreted as "Susceptibility" (*S*) genes encoding proteins in the host required for pathogen proliferation. Genes that encode such effector targets are promising candidates for genome editing; loss of function of the Pi21 HMA domain protein results in enhanced disease resistance. The role of HMA proteins in susceptibility remains to be established, but conceivably the metal-binding domain may influence host cell redox status, resulting in a more congenial environment for the pathogen.

Integrated decoy mechanisms have not been implicated in activation of mammalian NLRs. However, a conceptually similar mechanism appears

to underlie activation of mouse and rat NLRP1, an NLR that is activated by lethal factor, a virulence factor secreted by the anthrax bacterium *Bacillus anthracis* (*92*). Lethal factor is a protease that anthrax uses to degrade mitogen-activated protein kinases, kinases involved in host defense. As a countermeasure, certain rodent NLRP1 proteins are activated in response to direct cleavage by lethal factor. Cleavage results in removal of an N-terminal fragment of NLRP1 that normally holds NLRP1 in the "off" state, and cleavage of NLRP1 has been shown to be both necessary and sufficient to activate NLRP1B (*93*, *94*). Thus, the NLRP1 N terminus appears to behave like an integrated decoy domain, mimicking the cleavage site of the true effector targets. Unlike rodent NLRP1 proteins, human NLRP1 carries a pyrin domain at its N terminus. Interestingly, because NLRP1 signals via its C-terminal CARD, the N-terminal Pyrin domain is not required for signaling and currently is of unknown function (*95*). It is tempting to speculate that it serves as an integrated decoy to detect putative pyrin-targeting effector proteins.

Mining NLR repertoires and recognition strategies across taxa

Defining the repertoire of NLRs across species, or even across genotypes of the same species, requires an assessment of genetic diversity. We usually lack comprehensive catalogs of the diversity of NLRs present in species or strains of interest. Because NLR repertoire diversity is important for disease resistance in natural populations, definition of the pan-NLRome of any plant or animal species will catapult us beyond the limited understanding obtained by sequencing a single reference genome. In addition to the paired *NLR* genes described above, NLR-encoding genes typically exist as clustered families of closely related paralogs, or as true allelic series. Assembly of short-read whole-genome data often results in assembly errors in *NLR* loci. Sequence capture enables *NLR* gene enrichment sequencing (RenSeq) (*96*), and long-read DNA sequencing technology enables reads of complete NLRs to be obtained (*97*). Using biotinylated RNA probes designed to capture the repertoire of 450 *NLRs* predicted to be in the reference diploid potato genome, 750 NLRs were identified (*96*). These kinds of analyses on multiple accessions of plant species, combined with RenSeq applied to cDNA, will facilitate better insight into the repertoire of and genetic variation in NLRs, including those with integrated domains. Combined with mutagenesis, these methods accelerate isolation of useful resistance genes (*98*). Because integrated domains are envisaged as effector targets, a widespread understanding of their diversity will result in a broader understanding of the cellular processes usurped by pathogens (*43*). Plant genomes carry many genes encoding N- and C-terminal truncations of the canonical NLR structure, and these are also captured with RenSeq. Such "pieces" of NLRs do not fit the current mechanistic activation paradigm outlined above because many lack an active NBD. Nevertheless, their sequences are not degenerating into pseudogenes, and some are capable of signaling when overexpressed or mutated and are likely to contribute to overall NLR functional diversity (*47*, *99*).

Sequencing approaches such as RenSeq have not yet been applied to catalog animal NLR repertoires. One might anticipate that sequence capture methods would reveal considerable diversity in domain architectures as well as polymorphism, thus revealing some NLR families that are under diversifying selection and that are likely to be coevolving with pathogens in extremely large populations. Primitive vertebrates and invertebrates can carry large families of NLRs. For example, the coral *Acropora digitifera* carries ~500 NLRs, *Amphioxus* carries ~118 NLRs, and sea urchins carry ~203. There is no knowledge of the extent of diversity of NLRs in populations of these animals.

Mammals also display considerable diversity between species in their NLR repertoires. For example, unlike mice, the human reference genome appears to lack multiple NAIP paralogs, although it will be interesting to know whether this is true across all human populations. Short-read exome sequences are often difficult to assemble for paralogous and repetitive gene families; thus, RenSeq-type approaches could be valuable for assessing animal NLR diversity. Little is known about intraspecific variation in mammalian NLR repertoires, and sequence capture of NLRs could reveal diversity in innate immune surveillance capacity. Such insights could also be useful to engineer novel pathogen-resistance into animal species. For example, the NLRC4/NAIP alleles of appropriate strains of mice could enable enhanced resistance to *Salmonella* if expressed in transgenic chickens.

Toward synthetic detection platforms

Despite breakthroughs in our molecular understanding of NLR activation, knowledge of subsequent signaling steps and mechanisms remains weak. The pathways that connect NLR activation to outputs such as transcription of defense genes, changes in cell permeability, localized cell death, and systemic signaling remain poorly understood. Do activated, or dimerized, or oligomerized plant NLRs recruit new signaling proteins? How distinct are the signaling pathways controlled by the various N-terminal signaling domains recruited to the NLR chassis during evolution? Are integrated decoy domain NLRs modular? Can we engineer new or additional decoy domains into them to create or extend NLR function? As more structural and mechanistic information emerges on how plant and animal NLRs function, the engineering of novel, bespoke, and useful recognition capacities in plant and animal immune systems will become a more realistic goal.

REFERENCES AND NOTES

1. P. C. Ronald, B. Beutler, Plant and animal sensors of conserved microbial signatures. *Science* **330**, 1061–1064 (2010). doi: 10.1126/science.1189468; pmid: 21097929
2. F. Jacob, S. Vernaldi, T. Maekawa, Evolution and conservation of plant NLR functions. *Front. Immunol.* **4**, 297 (2013). doi: 10.3389/fimmu.2013.00297; pmid: 24093022
3. J. D. G. Jones, J. L. Dangl, The plant immune system. *Nature* **444**, 323–329 (2006). doi: 10.1038/nature05286; pmid: 17108957
4. E. Elinav, T. Strowig, J. Henao-Mejia, R. A. Flavell, Regulation of the antimicrobial response by NLR proteins. *Immunity* **34**, 665–679 (2011). doi: 10.1016/j.immuni.2011.05.007; pmid: 21616436
5. J. P.-Y. Ting *et al*., The NLR gene family: A standard nomenclature. *Immunity* **28**, 285–287 (2008). doi: 10.1016/j.immuni.2008.02.005; pmid: 18341998
6. M. Hamada *et al*., The complex NOD-like receptor repertoire of the coral *Acropora digitifera* includes novel domain combinations. *Mol. Biol. Evol.* **30**, 167–176 (2013). doi: 10.1093/molbev/mss213; pmid: 22936719
7. J. P. Rast, L. C. Smith, M. Loza-Coll, T. Hibino, G. W. Litman, Genomic insights into the immune system of the sea urchin. *Science* **314**, 952–956 (2006). doi: 10.1126/science.1134301; pmid: 17095692
8. S. Huang *et al*., Genomic analysis of the immune gene repertoire of amphioxus reveals extraordinary innate complexity and diversity. *Genome Res.* **18**, 1112–1126 (2008). doi: 10.1101/gr.069674.107; pmid: 18562681
9. K. Howe *et al*., Structure and evolutionary history of a large family of NLR proteins in the zebrafish. *Open Biol.* **6**, 160009 (2016). doi: 10.1098/rsob.160009; pmid: 27248802
10. M. Lamkanfi, V. M. Dixit, Mechanisms and functions of inflammasomes. *Cell* **157**, 1013–1022 (2014). doi: 10.1016/j.cell.2014.04.007; pmid: 24855941
11. A. Daskalov *et al*., Signal transduction by a fungal NOD-like receptor based on propagation of a prion amyloid fold. *PLOS Biol.* **13**, e1002059 (2015). doi: 10.1371/journal.pbio.1002059; pmid: 25671553
12. W. Dyrka *et al*., Diversity and variability of NOD-like receptors in fungi. *Genome Biol. Evol.* **6**, 3137–3158 (2014). doi: 10.1093/gbe/evu251; pmid: 25398782
13. S. Maekawa, U. Ohto, T. Shibata, K. Miyake, T. Shimizu, Crystal structure of NOD2 and its implications in human disease. *Nat. Commun.* **7**, 11813 (2016). doi: 10.1038/ncomms11813; pmid: 27283905
14. V. A. K. Rathinam, K. A. Fitzgerald, Inflammasome complexes: Emerging mechanisms and effector functions. *Cell* **165**, 792–800 (2016). doi: 10.1016/j.cell.2016.03.046; pmid: 27153493
15. D. Sharma, T.-D. Kanneganti, The cell biology of inflammasomes: Mechanisms of inflammasome activation and regulation. *J. Cell Biol.* **213**, 617–629 (2016). doi: 10.1083/jcb.201602089; pmid: 27325789
16. R. Caruso, N. Warner, N. Inohara, G. Núñez, NOD1 and NOD2: Signaling, host defense, and inflammatory disease. *Immunity* **41**, 898–908 (2014). doi: 10.1016/j.immuni.2014.12.010; pmid: 25526305
17. Z. Duxbury *et al*., Pathogen perception by NLRs in plants and animals: Parallel worlds. *BioEssays* **38**, 769–781 (2016). doi: 10.1002/bies.201600046; pmid: 27339076
18. H. Wen, E. A. Miao, J. P.-Y. Ting, Mechanisms of NOD-like receptor-associated inflammasome activation. *Immunity* **39**, 432–441 (2013). doi: 10.1016/j.immuni.2013.08.037; pmid: 24054327
19. A. Bentham, H. Burdett, P. A. Anderson, S. J. Williams, B. Kobe, Animal NLRs provide structural insights into plant NLR function. *Ann. Bot.* mcw171 (2016). doi: 10.1093/aob/mcw171; pmid: 27562749
20. D. D. Leipe, E. V. Koonin, L. Aravind, STAND, a class of P-loop NTPases including animal and plant regulators of programmed cell death: Multiple, complex domain architectures, unusual phyletic patterns, and evolution by horizontal gene transfer. *J. Mol. Biol.* **343**, 1–28 (2004). doi: 10.1016/j.jmb.2004.08.023; pmid: 15381417
21. J. E. Walker, M. Saraste, M. J. Runswick, N. J. Gay, Distantly related sequences in the alpha- and beta-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* **1**, 945–951 (1982). pmid: 6329717
22. E. V. Koonin, L. Aravind, The NACHT family—A new group of predicted NTPases implicated in apoptosis and MHC transcription activation. *Trends Biochem. Sci.* **25**, 223–224 (2000). doi: 10.1016/S0968-0004(00)01577-2; pmid: 10782090
23. E. A. van der Biezen, J. D. G. Jones, The NB-ARC domain: A novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. *Curr. Biol.* **8**, R226–R227 (1998). doi: 10.1016/S0960-9822(98)70145-9; pmid: 9545207

24. L. Aravind, V. M. Dixit, E. V. Koonin, The domains of death: Evolution of the apoptosis machinery. *Trends Biochem. Sci.* **24**, 47–53 (1999). doi: 10.1016/S0968-0004(98)01341-3; pmid: 10098397
25. E. V. Koonin, L. Aravind, Origin and evolution of eukaryotic apoptosis: The bacterial connection. *Cell Death Differ.* **9**, 394–404 (2002). doi: 10.1038/sj.cdd.4400991; pmid: 11965492
26. M. Albrecht, F. L. W. Takken, Update on the domain architectures of NLRs and R proteins. *Biochem. Biophys. Res. Commun.* **339**, 459–462 (2006). doi: 10.1016/j.bbrc.2005.10.074; pmid: 16271351
27. J.-X. Yue, B. C. Meyers, J.-Q. Chen, D. Tian, S. Yang, Tracing the origin and evolutionary history of plant nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes. *New Phytol.* **193**, 1049–1063 (2012). doi: 10.1111/j.1469-8137.2011.04006.x; pmid: 22212278
28. K. S. Kobayashi, P. J. van den Elsen, NLRC5: A key regulator of MHC class I-dependent immune responses. *Nat. Rev. Immunol.* **12**, 813–820 (2012). doi: 10.1038/nri3339; pmid: 23175229
29. J. P. Y. Ting, J. A. Duncan, Y. Lei, How the noninflammasome NLRs function in the innate immune system. *Science* **327**, 286–290 (2010). doi: 10.1126/science.1184004; pmid: 20075243
30. S. Yuan, C. W. Akey, Apoptosome structure, assembly, and procaspase activation. *Structure* **21**, 501–515 (2013). doi: 10.1016/j.str.2013.02.024; pmid: 23561633
31. J. L. Dangl, D. M. Horvath, B. J. Staskawicz, Pivoting the plant immune system from dissection to deployment. *Science* **341**, 746–751 (2013). doi: 10.1126/science.1236011; pmid: 23950531
32. M. Bernoux *et al*., Comparative analysis of the flax immune receptors L6 and L7 suggests an equilibrium-based switch activation model. *Plant Cell* **28**, 146–159 (2016). pmid: 26744216
33. O. Danot, E. Marquenet, D. Vidal-Ingigliardi, E. Richet, Wheel of Life, Wheel of death: A mechanistic insight into signaling by STAND proteins. *Structure* **17**, 172–182 (2009). doi: 10.1016/j.str.2009.01.001; pmid: 19217388
34. A. Lu *et al*., Unified polymerization mechanism for the assembly of ASC-dependent inflammasomes. *Cell* **156**, 1193–1206 (2014). doi: 10.1016/j.cell.2014.02.008; pmid: 24630722
35. X. Cai *et al*., Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. *Cell* **156**, 1207–1222 (2014). doi: 10.1016/j.cell.2014.01.063; pmid: 24630723
36. Z. Hu *et al*., Crystal structure of NLRC4 reveals its autoinhibition mechanism. *Science* **341**, 172–175 (2013). doi: 10.1126/science.1236381; pmid: 23765277
37. H. M. Hoffman, J. L. Mueller, D. H. Broide, A. A. Wanderer, R. D. Kolodner, Mutation of a new gene encoding a putative pyrin-like protein causes familial cold autoinflammatory syndrome and Muckle-Wells syndrome. *Nat. Genet.* **29**, 301–305 (2001). doi: 10.1038/ng756; pmid: 11687797
38. H. Yang *et al*., A mutant CHS3 protein with TIR-NB-LRR-LIM domains modulates growth, cell death and freezing tolerance in a temperature-dependent manner in *Arabidopsis*. *Plant J.* **63**, 283–296 (2010). doi: 10.1111/j.1365-313X.2010.04241.x; pmid: 20444230
39. P. Mestre, D. C. Baulcombe, Elicitor-mediated oligomerization of the tobacco N disease resistance protein. *Plant Cell* **18**, 491–501 (2006). doi: 10.1105/tpc.105.037234; pmid: 16387833
40. K. J. Schreiber, A. Bentham, S. J. Williams, B. Kobe, B. J. Staskawicz, Multiple domain associations within the *Arabidopsis* immune receptor RPP1 regulate the activation of programmed cell death. *PLOS Pathog.* **12**, e1005769 (2016). doi: 10.1371/journal.ppat.1005769; pmid: 27427964
41. R. A. L. van der Hoorn, S. Kamoun, From guard to decoy: A new model for perception of plant pathogen effectors. *Plant Cell* **20**, 2009–2017 (2008). doi: 10.1105/tpc.108.060194; pmid: 18723576
42. S. Césari, M. Bernoux, P. Moncuquet, T. Kroj, P. N. Dodds, A novel conserved mechanism for plant NLR protein pairs: The "integrated decoy" hypothesis. *Front. Plant Sci.* **5**, 606 (2014). doi: 10.3389/fpls.2014.00606; pmid: 25506347
43. P. F. Sarris, V. Cevik, G. Dagdas, J. D. G. Jones, K. V. Krasileva, Comparative analysis of plant immune receptor architectures uncovers host proteins likely targeted by pathogens. *BMC Biol.* **14**, 8 (2016). doi: 10.1186/s12915-016-0228-7; pmid: 26891798
44. T. Kroj, E. Chanclud, C. Michel-Romiti, X. Grand, J.-B. Morel, Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread. *New Phytol.* **210**, 618–626 (2016). doi: 10.1111/nph.13869; pmid: 26848538
45. F. M. Ausubel, Are innate immune signaling pathways in plants and animals conserved? *Nat. Immunol.* **6**, 973–979 (2005). doi: 10.1038/ni1253; pmid: 16177805
46. C.-H. Wu, K. Belhaj, T. O. Bozkurt, M. S. Birk, S. Kamoun, Helper NLR proteins NRC2a/b and NRC3 but not NRC1 are required for Pto-mediated cell death and resistance in Nicotiana benthamiana. *New Phytol.* **209**, 1344–1352 (2016). doi: 10.1111/nph.13764; pmid: 26592988
47. V. Bonardi, K. Cherkis, M. T. Nishimura, J. L. Dangl, A new eye on NLR proteins: Focused on clarity or diffused by complexity? *Curr. Opin. Immunol.* **24**, 41–50 (2012). doi: 10.1016/j.coi.2011.12.006; pmid: 22305607
48. E. M. Kofoed, R. E. Vance, Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature* **477**, 592–595 (2011). doi: 10.1038/nature10394; pmid: 21874021
49. Y. Zhao *et al*., The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature* **477**, 596–600 (2011). doi: 10.1038/nature10510; pmid: 21918512
50. Z. Hu *et al*., Structural and biochemical basis for induced self-propagation of NLRC4. *Science* **350**, 399–404 (2015). doi: 10.1126/science.aac5489; pmid: 26449475
51. L. Zhang *et al*., Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. *Science* **350**, 404–409 (2015). doi: 10.1126/science.aac5789; pmid: 26449474
52. S. M. Collier, L.-P. Hamel, P. Moffett, Cell death mediated by the N-terminal domains of a unique and highly conserved class of NB-LRR protein. *Mol. Plant Microbe Interact.* **24**, 918–931 (2011). doi: 10.1094/MPMI-03-11-0050; pmid: 21501087
53. J. R. Peart, P. Mestre, R. Lu, I. Malcuit, D. C. Baulcombe, NRG1, a CC-NB-LRR protein, together with N, a TIR-NB-LRR protein, mediates resistance against tobacco mosaic virus. *Curr. Biol.* **15**, 968 (2005).
54. V. Bonardi *et al*., Expanded functions for a family of plant intracellular immune receptors beyond specific recognition of pathogen effectors. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 16463–16468 (2011). doi: 10.1073/pnas.1113726108; pmid: 21911370
55. N. Hayashi *et al*., Durable panicle blast-resistance gene Pb1 encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J.* **64**, 498–510 (2010). doi: 10.1111/j.1365-313X.2010.04348.x; pmid: 20807214
56. V. Bonardi, J. L. Dangl, How complex are intracellular immune receptor signaling complexes? *Front. Plant Sci.* **3**, 237 (2012). doi: 10.3389/fpls.2012.00237; pmid: 23109935
57. S. J. Williams *et al*., Structural basis for assembly and function of a heterodimeric plant immune receptor. *Science* **344**, 299–303 (2014). doi: 10.1126/science.1247357; pmid: 24744375
58. J. G. Ellis, P. N. Dodds, G. J. Lawrence, Flax rust resistance gene specificity is based on direct resistance-avirulence protein interactions. *Annu. Rev. Phytopathol.* **45**, 289–306 (2007). doi: 10.1146/annurev.phyto.45.062806.094331; pmid: 17430087
59. E. A. Van der Biezen, J. D. Jones, Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* **23**, 454–456 (1998). doi: 10.1016/S0968-0004(98)01311-5; pmid: 9868361
60. J. L. Dangl, J. D. Jones, Plant pathogens and integrated defence responses to infection. *Nature* **411**, 826–833 (2001). doi: 10.1038/35081161; pmid: 11459065
61. E.-H. Chung *et al*., Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. *Cell Host Microbe* **9**, 125–136 (2011). doi: 10.1016/j.chom.2011.01.009; pmid: 21320695
62. E.-H. Chung, F. El-Kasmi, Y. He, A. Loehr, J. L. Dangl, A plant phosphoswitch platform repeatedly targeted by type III effector proteins regulates the output of both tiers of plant immune receptors. *Cell Host Microbe* **16**, 484–494 (2014). doi: 10.1016/j.chom.2014.09.004; pmid: 25299334
63. J. Liu, J. M. Elmore, Z.-J. D. Lin, G. Coaker, A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. *Cell Host Microbe* **9**, 137–146 (2011). doi: 10.1016/j.chom.2011.01.010; pmid: 21320696
64. D. Lee, G. Bourdais, G. Yu, S. Robatzek, G. Coaker, Phosphorylation of the plant immune regulator RPM1-INTERACTING PROTEIN4 enhances plant plasma membrane H^+-ATPase activity and inhibits flagellin-triggered immune responses in *Arabidopsis*. *Plant Cell* **27**, 2042–2056 (2015). doi: 10.1105/tpc.114.132308; pmid: 26198070
65. M. J. Axtell, B. J. Staskawicz, Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. *Cell* **112**, 369–377 (2003). doi: 10.1016/S0092-8674(03)00036-9; pmid: 12581526
66. D. Mackey, B. F. Holt 3rd, A. Wiig, J. L. Dangl, RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. *Cell* **108**, 743–754 (2002). doi: 10.1016/S0092-8674(02)00661-X; pmid: 11955429
67. F. Shao *et al*., Cleavage of Arabidopsis PBS1 by a bacterial type III effector. *Science* **301**, 1230–1233 (2003). doi: 10.1126/science.1085671; pmid: 12947197
68. J. Ade, B. J. DeYoung, C. Golstein, R. W. Innes, Indirect activation of a plant nucleotide binding site-leucine-rich repeat protein by a bacterial protease. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 2531–2536 (2007). doi: 10.1073/pnas.0608779104; pmid: 17277084
69. S. H. Kim, D. Qi, T. Ashfield, M. Helm, R. W. Innes, Using decoys to expand the recognition specificity of a plant disease resistance protein. *Science* **351**, 684–687 (2016). doi: 10.1126/science.aad3436; pmid: 26912853
70. O. Rowland *et al*., Functional analysis of Avr9/Cf-9 rapidly elicited genes identifies a protein kinase, ACIK1, that is essential for full Cf-9-dependent disease resistance in tomato. *Plant Cell* **17**, 295–310 (2005). doi: 10.1105/tpc.104.026013; pmid: 15598806
71. V. Ntoukakis, I. M. Saur, B. Conlan, J. P. Rathjen, The changing of the guard: The Pto/Prf receptor complex of tomato and pathogen recognition. *Curr. Opin. Plant Biol.* **20**, 69–74 (2014). doi: 10.1016/j.pbi.2014.04.002; pmid: 24845576
72. G. Wang *et al*., The decoy substrate of a pathogen effector and a pseudokinase specify pathogen-induced modified-self recognition and immunity in plants. *Cell Host Microbe* **18**, 285–295 (2015). doi: 10.1016/j.chom.2015.08.004; pmid: 26355215
73. N. Inohara *et al*., Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J. Biol. Chem.* **278**, 5509–5512 (2003). doi: 10.1074/jbc.C200673200; pmid: 12514169
74. M. Chamaillard *et al*., An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. *Nat. Immunol.* **4**, 702–707 (2003). doi: 10.1038/ni945; pmid: 12796777
75. S. E. Girardin *et al*., Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. *Science* **300**, 1584–1587 (2003). doi: 10.1126/science.1084677; pmid: 12791997
76. A. M. Keestra *et al*., Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1. *Nature* **496**, 233–237 (2013). doi: 10.1038/nature12025; pmid: 23542589
77. A. M. Keestra-Gounder *et al*., NOD1 and NOD2 signalling links ER stress with inflammation. *Nature* **532**, 394–397 (2016). doi: 10.1038/nature17631; pmid: 27007849
78. Y. He, M. Y. Zeng, D. Yang, B. Motro, G. Núñez, NEK7 is an essential mediator of NLRP3 activation downstream of potassium efflux. *Nature* **530**, 354–357 (2016). doi: 10.1038/nature16959; pmid: 26814970
79. H. Shi *et al*., NLRP3 activation and mitosis are mutually exclusive events coordinated by NEK7, a new inflammasome component. *Nat. Immunol.* **17**, 250–258 (2016). doi: 10.1038/ni.3333; pmid: 26642356
80. J. L. Schmid-Burgk *et al*., A Genome-wide CRISPR (clustered regularly interspaced short palindromic repeats) screen identifies NEK7 as an essential component of NLRP3 inflammasome activation. *J. Biol. Chem.* **291**, 103–109 (2016). doi: 10.1074/jbc.C115.700492; pmid: 26553871
81. K. Bomblies *et al*., Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *PLOS Biol.* **5**, e236 (2007). doi: 10.1371/journal.pbio.0050236; pmid: 17803357
82. E. Chae *et al*., Species-wide genetic incompatibility analysis identifies immune genes as hot spots of deleterious epistasis. *Cell* **159**, 1341–1351 (2014). doi: 10.1016/j.cell.2014.10.049; pmid: 25467443
83. S. Césari *et al*., The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. *EMBO J.* **33**, 1941–1959 (2014). doi: 10.15252/embj.201487923; pmid: 25024433

84. M. Narusaka *et al*., RRS1 and RPS4 provide a dual Resistance-gene system against fungal and bacterial pathogens. *Plant J.* **60**, 218–226 (2009). doi: 10.1111/j.1365-313X.2009.03949.x; pmid: 19519800
85. N. Ishihama, H. Yoshioka, Post-translational regulation of WRKY transcription factors in plant immunity. *Curr. Opin. Plant Biol.* **15**, 431–437 (2012). doi: 10.1016/j.pbi.2012.02.003; pmid: 22425194
86. P. F. Sarris *et al*., A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell* **161**, 1089–1100 (2015). doi: 10.1016/j.cell.2015.04.024; pmid: 26000484
87. C. Le Roux *et al*., A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. *Cell* **161**, 1074–1088 (2015). doi: 10.1016/j.cell.2015.04.025; pmid: 26000483
88. M. Narusaka *et al*., Interfamily transfer of dual NB-LRR genes confers resistance to multiple pathogens. *PLOS ONE* **8**, e55954–e55958 (2013). doi: 10.1371/journal.pone.0055954; pmid: 23437080
89. R. Weßling *et al*., Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. *Cell Host Microbe* **16**, 364–375 (2014). doi: 10.1016/j.chom.2014.08.004; pmid: 25211078
90. A. Maqbool *et al*., Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. *eLife* **4**, 213 (2015). doi: 10.7554/eLife.08709; pmid: 26304198
91. S. Fukuoka *et al*., Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* **325**, 998–1001 (2009). doi: 10.1126/science.1175550; pmid: 19696351
92. E. D. Boyden, W. F. Dietrich, Nalp1b controls mouse macrophage susceptibility to anthrax lethal toxin. *Nat. Genet.* **38**, 240–244 (2006). doi: 10.1038/ng1724; pmid: 16429160
93. J. Chavarría-Smith, R. E. Vance, Direct proteolytic cleavage of NLRP1B is necessary and sufficient for inflammasome activation by anthrax lethal factor. *PLOS Pathog.* **9**, e1003452 (2013). doi: 10.1371/journal.ppat.1003452; pmid: 23818853
94. J. L. Levinsohn *et al*., Anthrax lethal factor cleavage of Nlrp1 is required for activation of the inflammasome. *PLOS Pathog.* **8**, e1002638 (2012). doi: 10.1371/journal.ppat.1002638; pmid: 22479187
95. J. N. Finger *et al*., Autolytic proteolysis within the function to find domain (FIIND) is required for NLRP1 inflammasome activity. *J. Biol. Chem.* **287**, 25030–25037 (2012). doi: 10.1074/jbc.M112.378323; pmid: 22665479
96. F. Jupe *et al*., Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. *Plant J.* **76**, 530–544 (2013). doi: 10.1111/tpj.12307; pmid: 23937694
97. K. Witek *et al*., Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT sequencing. *Nat. Biotechnol.* **34**, 656–660 (2016). doi: 10.1038/nbt.3540; pmid: 27111721
98. B. Steuernagel *et al*., Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. *Nat. Biotechnol.* **34**, 652–655 (2016). doi: 10.1038/nbt.3543; pmid: 27111722
99. R. S. Nandety *et al*., The role of TIR-NBS and TIR-X proteins in plant basal defense responses. *Plant Physiol.* **162**, 1459–1472 (2013). doi: 10.1104/pp.113.219162; pmid: 23735504

ACKNOWLEDGMENTS

NLR and effector research in the laboratories of the authors is funded by the Biotechnology and Biological Sciences Research Council, European Resdearch Council, Gordon and Betty Moore Foundation (GBMF), and the Gatsby Foundation (J.D.G.J.) and the NSF, NIH (J.L.D. and R.E.V.), GMBF (J.L.D.), and HHMI (J.L.D. and R.E.V.). J.D.G.J. thanks B. Staskawicz for hosting a brief sabbatical visit, during which conversations leading to this review were initiated, and we thank A. Daskalov and O. Furzer for helpful discussion. We thank V. Maltez, E. Miao, I. Rauch, K. Witek, and J. Parker for providing unpublished images for Fig. 1 and J. Tenthorey for help with Fig. 2.

10.1126/science.aaf6395