boundary attributed to increased chromatin interactions (Narendra et al., 2015). While it is still unclear how exactly CTCF contributes to formation or maintenance of TAD boundaries, its ubiquitous expression pattern and high degree of protein sequence conservation help to explain the stable TADs structure in different cell types and species.

The newly reported findings demonstrate that inversions, deletions, or other structural variations that affect TAD boundaries can change chromatin organization, rewire enhancer-promoter interactions, alter gene expression patterns, and cause human diseases. As more and more structural variants are discovered in the human genome and are linked to uncharacterized genetic disorders, consideration of their impact on chromatin topology will be essential for understanding their molecular mechanisms of pathogenesis.

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Treasure Your Exceptions: Unusual Domains in Immune Receptors Reveal Host Virulence Targets

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A mechanistic understanding of how plant pathogens modulate their hosts is critical for rationally engineered disease resistance in agricultural systems. Two new studies show that genomically paired plant immune receptors have incorporated decoy domains that structurally mimic pathogen virulence targets to monitor attempted host immunosuppression.

Humans have been manipulating genes encoding plant immune system receptors for a hundred years. Our foods reflect the immense success of Mendelian genetics, now coupled with genomics-based technologies, in the hands of plant breeders. However, compare that century of human opportunity to the millennia that pathogenic microbes have had to target and suppress critical components of the plant immune system, allowing them to evade host recognition, proliferate, and cause disease. The resulting arsenal of virulence proteins independently evolved in microbes as diverse as bacteria, fungi, oomycetes, and nematodes. This evolutionary tug of war presents ongoing challenges for plant breeders, who must continually identify new genetic sources of useful immune receptors, and fantastic opportunities for molecular biologists to accelerate our mechanistic understanding of host immunity. In this issue of *Cell*, two groups Le Roux et al. (2015 and Sarris et al. (2015) dissect the mechanism by which plant effectors activate an unusual class of immune receptor. In doing so, they reveal a hidden genomic signature that facilitates definition of novel host targets across the plant phylogeny.

Lacking an adaptive immune system and circulating immunocytes, plants rely on an elaborate innate immune system to defend themselves against pathogens. The plant immune system can be divided into two tiers (Jones and Dangl, 2006). The first contains receptors able to recognize several classes of

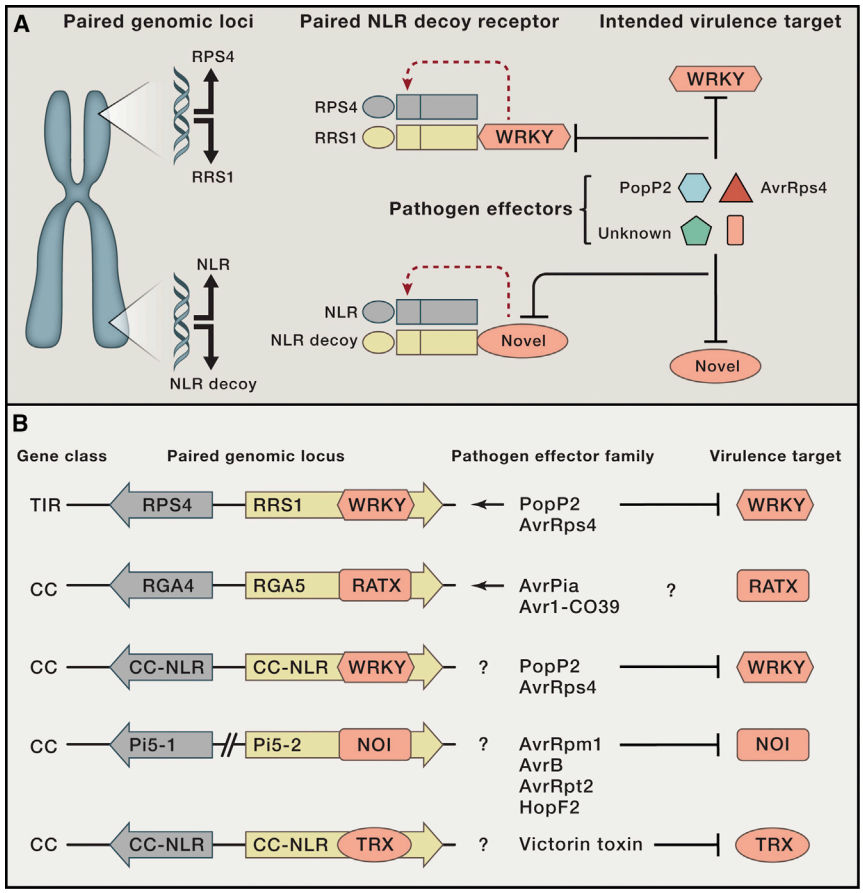

Figure 1. NLR Immune Receptors with Unusual Domains Reveal Novel Virulence Targets

(A) NLR immune receptors with unusual domains reveal novel virulence targets. (Top) Paired NLR fused decoy pairs, like *Arabidopsis* RPS4/RRS1, exist at various genomic loci. Pathogen effectors target host proteins in order to promote virulence. RPS4/RRS1 intercepts effectors via the WRKY decoy domain in RRS1. Effector modulation of decoy domains activates NLR pairs (red dashed arrow), triggering disease resistance responses. Identification of novel domains attached to NLRs, especially pairs, will facilitate the identification of novel virulence targets.

(B) Plausible NLR-decoy fusion proteins exist in the rice genome. Each example is detailed in the text.

conserved microbe-associated molecular patterns (MAMPs). These receptors, typically plasma membrane receptor-like kinases (RLKs), detect extracellular MAMPs and activate defense responses to ward off most microbes. A common strategy for many plant pathogens is to deliver effector proteins in order to suppress RLK-dependent immunity. In response, plants have evolved a second tier of intracellular receptors, called NLRs. Canonical plant NLR proteins are comprised of N-terminal Toll/interleukin 1 receptor (TIR) or coiled-coil (CC) domains attached to a central nucleotide-binding domain and a C-terminal leucine-rich repeat. NLRs are activated by direct binding of a pathogen effector protein, or by recognition of the product of effector action on its intended host target, or a molecular decoy of that target. NLR activation drives a powerful immune response, epistatic to effector-mediated immunosuppression and sufficient to limit pathogen spread. Unusual domains have been found fused to NLR genes, but until now their function has remained obscure (Cesari et al., 2014). Sarris et al. and Le Roux et al. elegantly demonstrate that the unusual WRKY transcription factor-like domain fused to the *Arabidopsis thaliana* RRS1 TIR-NLR is actually a "fused decoy" that intercepts pathogen effectors as they attempt to target WRKY transcription factors to suppress immune transcriptional outputs and thus promote pathogen virulence.

RRS1 and RPS4 are part of a "paired decoy NLR system" defined by genetic, biochemical, and phenotypic evidence (Figure 1A) (1) RPS4 and RRS1 alleles are very tightly linked, encoded in a head-to-head arrangement with a short, shared promoter; (2) the two proteins interact with each other and form hetero-multimers (Williams et al., 2014); and (3) both are genetically co-required to recognize two known bacterial effectors and one unidentified fungal effector protein (Williams et al., 2014).

Working the WRKY Domain

While RPS4 and RRS1 are both TIR-NLR proteins, the most obvious difference between them is the C-terminal WRKY domain fused to RRS1. The functions of the WRKY domain in RRS1 remained a matter of speculation for years, but Sarris et al. and Le Roux et al. demonstrate that the RPS4/RRS1 pair uses this decoy domain to hijack effectors that have evolved to target WRKY transcription factors, a large class of plant-specific and often immune functional transcription factors (Rushton et al., 2010). Additionally, RRS1 alleles feature variable and functional C-terminal-extensions beyond the WRKY domain. The RRS1-R allele requires a 96 amino acid extension that is fused to its WRKY domain for activation by the *Ralstonia solanacearum* effector PopP2; the RRS1-S allele carries the WRKY fusion, but not the extension, and is not activated by PopP2.

PopP2 is a YopJ family effector with acetyl-transferase activity. Interaction of this effector with both RRS1-S and RRS1-R was previously described to occur in the nucleus of *A. thaliana* and *N. benthamiana* cells. PopP2 interaction with RRS1-R leads to immune activation, but importantly this requires RPS4. On the other hand, the *Pseudomonas syringae* pv. *pisi* effector AvrRps4 is recognized through RRS1-R and RRS-S, also in an RPS4-dependent manner (Williams et al., 2014).

Collectively, both studies validate PopP2- and AvrRps4-RRS1 interactions. Domain swapping of the bacterial DNA-binding LexA protein for the RRS1 WRKY domain demonstrated that the WRKY domain is required for interaction

with both PopP2 and AvrRps4. However, AvrRps4 retains residual binding to RRS1-R truncations lacking the WRKY domain. How these two effectors exhibit different requirements for activating the same NLR pair will tell us much about how NLRs are or are not evolutionarily constrained.

PopP2 Acetylates WRKY Domains

Sarris et al. and Le Roux et al. show that PopP2 acetylates lysines on the highly conserved DNA-binding WRKTGQK sequence in both RRS1-R and RRS1-S. According to Sarris and collaborators, two lysines (K1217 and K1221) occur in the RRS1 WRKYGQK motif and are required for DNA-binding. Le Roux et al. use a proteomics approach to show that PopP2-dependent K1221-acetylation might be the predominant event and that acetylation of the two Lys residues in the WRKYGQK motif inhibits RRS1 DNA-binding to the previously defined W-box *cis* DNA element in vitro and in vivo.

The introduction of a K1221R mutation in RRS1-R dampens immune response by preventing interaction with, and acetylation by, PopP2. Recognition of AvrRps4 is also lost in RRS1-R K1221R mutants. In contrast, transiently expressed RRS1-R K1221Q mutants, which structurally mimic lysine acetylation, exhibit RPS4-dependent auto-activation. These results indicate that despite differing requirements for activation, there is mechanistic overlap in PopP2 and AvrRps4 function at the critical WRKY DNA-binding surface that results in activation of RPS4. Accordingly, Sarris et al. show that in mixed innoculations, *Pseudomonas fluorescens* delivery of PopP2 restores full growth of a *Pseudomonas syringae* DC3000 strain normally limited by its expression of AvrRps4. This is consistent with PopP2-dependent acetylation of RRS1 compromising the ability of the RRS1/RPS4 complex to recognize and be activated by AvrRps4.

Why has natural selection favored the fusion of a WRKY domain to RRS1? There over 70 WRKY TFs in *Arabidopsis thaliana*, which function as activators or repressors in a plethora of plant processes including biotic stress (Rushton et al., 2010). Together, the authors show that both AvrRps4 and PopP2 interact with up to 14 WRKY transcription factors, of which 12 are acetylated by PopP2. Acetylation of the K1221 equivalent interferes with DNA binding in the case of immune-related WRKY22, WRKY53 and the flagellin-induced WRKY41. Instead of evolution of one or more NLR receptors to monitor the biochemical integrity of the WRKY domains of all these proteins, natural selection has favored the fusion of a WRKY domain to its own receptor, giving rise to RRS1. An explicit prediction from this interpretation is that the affinity of PopP2 is higher for the RRS1 WRKY domain than for other WRKY domains in vivo. This is analogous to the Pto/Fen family of kinases that intercept effectors bound for the kinase domains of important RLKs as decoys, and transduce that interception to an NLR encoded nearby, and physically associated, but in the absence of a domain fusion (Ntoukakis et al., 2013).

Decoy Domains Are a Shortcut to Defining Novel Virulence Targets

Paired NLRs with fused decoy domains, while rare in any single genome, are a general feature of the immune receptor repertoire across all plants (Figure 1B; Cesari et al., 2014). The RPS4/RRS1-WRKY pairs are found at the tips of part of the Brassicaceae, present in *Arabidopsis* accessions, and its relative *Capsella rubella*. However, this general NLR evolutionary innovation is widespread. The rice RGA4/RGA5 NLR pair encodes CC-NLRs, one fused to an effector-targeted decoy domain called RATX, a putative metal-binding domain found in other rice proteins (Cesari et al., 2014). Hence both monocots and dicots deploy the paired fused decoy strategy, and both the TIR and CC classes of NLRs can be recruited into these.

Beyond RGA4/RGA5, casual inspection of the rice genome reveals additional putative NLR pairs, some featuring decoy domains related to proteins with immune functions that are targeted by effectors in other plant species. Hence, these fused decoy pairs are plausibly functional, and predict the independent evolution of effectors from pathogens of diverse host plants that activate them (Figure 1B). A predicted rice CC-NLR-WRKY decoy fusion is analogous to RRS1. The rice Pi-ta paired NLR contains a thioredoxin domain. Thus, Pi-ta could function as a decoy for pathogen molecules that target thioredoxin, such as the *Cochliobolus victoriae* fungal toxin victorin (Sweat and Wolpert, 2007). Of particular interest to us, the rice genome also contains two independently derived NLR pairs where one member carries a putative NOI domain decoy fusion. In *Arabidopsis*, the NOI domain of RIN4 is targeted by at least four sequence unrelated *Pseudomonas syringae* effectors and different flavors of biochemical modification of RIN4 by these effectors activates at least two different CC-NLR proteins (Jones and Dangl, 2006). These pairs contain binding and/or cleavage sites for two of the effectors targeting RIN4 and thus present plausible decoys for effector classes that target RIN4.

NLR pairs and their fused decoy domains present a ripe opportunity to broaden our knowledge of host virulence targets. Because many independently evolved pathogen effectors converge on a limited set of host targets (Mukhtar et al., 2011), a specific hypothesis is that NLR pairs will incorporate domains from these convergent targets as convergent decoys. There are numerous NLRs that contain novel domains, many of which are undescribed. As we extend from these well-studied examples, will we converge onto a theme? Are all unusual domains incorporated into plant NLR receptors true decoys? Or are they domains that extend the functional flexibility of NLRs once activated by effector detection? We favor the former, and propose that the set of hypothetical decoy domains that exist across the plant phylogeny will be highly enriched for effector targets. Thus, they provide a shortcut to identification of novel host proteins and important immune system components. As Bateson counseled, "Treasure your exceptions! When there are none, the work gets so dull that no one cares to carry it further. Keep them always uncovered and in sight. Exceptions are like the rough brickwork of a growing building which tells that there is more to come and shows where the next construction is to be" (Bateson, 1908).

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Deciphering the Tubulin Code

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Enzymes of the tubulin tyrosine ligase-like (TTLL) family posttranslationally modify and thereby mark microtubules by glutamylation, generating specific recognition sites for microtubule-interacting proteins. Garnham et al. report the first structure of a TTLL protein alone and in complex with microtubules, elucidating their mechanism of action.

Markers, flags, and signs are commonly used in our daily lives. Rangers mark trees in the forest so that wood workers know which tree is left in place and which one to cut. Barcodes on supermarket products carry hidden messages like the price, the destination of the product, or its best-before date. Street signs show us which street we are driving on, where it is leading, and how fast we are allowed to drive. Knowing the meaning of these symbols, we decode hidden information and make use of it. Biology also utilizes codes, and in the case of microtubules, posttranslational modifications (PTM) mark subpopulations and modify the interactions with microtubule effectors (Janke, 2014). This so-called "tubulin code" (Verhey and Gaertig, 2007) is established by detyrosination, glutamylation, glycylation, acetylation, phosphorylation, palmitoylation, and generation of Δ2-tubulin (Westermann and Weber, 2003) (Figure 1A). With the exception of acylation and palmitoylation, these modifications occur at the flexible C-terminal tails of α- and β-tubulin protruding from the surface of microtubules. Microtubule PTMs alter the interaction with microtubule-associated proteins (MAPs), motor proteins such as kinesin and dynein, and plus-end tracking proteins (+TIPs) and are therefore essential for intracellular trafficking, assembly and motility of cilia, microtubule dynamics, and mitosis. Dysfunction of microtubule PTM enzymes has detrimental effects for the organism, leading to developmental disorders and neurodegenerative diseases. Bacteria also make use of the tubulin code and specifically modify microtubules of the host. Toxin A from *Clostridium difficile*, for example, decreases acetylation of tubulin and thereby causes acute inflammation (Nam et al., 2010).

Due to missing structural information on tubulin PTM enzymes in complex with microtubules, it has so far not been apparent how these enzymes specifically recognize and modify the microtubule—that is, how they establish the "tubulin code." In this issue of *Cell*, the teams around Antonina Roll-Mecak, Ron Milligan, and Gabe Lander present a structural explanation for the specific binding and modification of microtubules by tubulin tyrosine ligase-like enzyme 7 (TTLL7) (Garnham et al., 2015), which is responsible for the ATP-dependent initiation and elongation of polyglutamylation of microtubules (Mukai et al., 2009).

The crystal structure of TTLL7 shows that its active site has the same ATP-grasp ligase fold found in the homologous tubulin tyrosine ligase (TTL) (Janke et al., 2005; Szyk et al., 2011). However, c-MTBD, a major positively charged domain of the protein that is not found in TTL, is not resolved in the structure. Interestingly, this domain is ordered in TTLL7 when bound to the microtubule, interacting with a negatively charged patch on α-tubulin (Figure 1B). The authors show convincingly that the positive charge is essential for proper binding not only of TTLL7, but also of other members of the TTLL glutamylases, even if the fold of the domain is probably not conserved.

The cryo-EM structure of the TTLL7-microtubule complex, which represents the first structure of a microtubule PTM enzyme in complex with its substrate, also reveals additional densities corresponding to the α- and β-C-terminal tails