How to trick a plant pathogen?

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Plants can get sick too. In fact, they get infected by all types of microbes and little critters. But plants have evolved an effective immune system to fight off pathogen invasion. Amazingly, nearly every single plant cell is able to protect itself and its neighbours against infections. The plant immune system gets switched on when one of its many immune receptors matches a ligand in the pathogen. As a consequence of a long evolutionary history of fighting off pathogens, immune receptors are now encoded by hundreds of genes that populate the majority of plant genomes. Understanding how the plant immune system functions and how it has evolved can give invaluable insights that would benefit modern agriculture and help breeding disease resistant crops.

Anyone who has kept a garden or worked in a farm knows that plants can get sick. They get infected by all types of microbes and pests. Plants can even be infected by other plants—the parasitic type. But plants don't sit idle in the face of their biotic foes. They have an immune system to fight off invading pathogens and pests. In fact, an old plant pathology adage postulates that most plants are resistant to most pathogens. As far as plants go, immunity is the norm and disease is the exception. Otherwise, our planet wouldn't be so green and lush with plant life.

The plant immune system is very effective. A first layer of defense is formed by an arsenal of immune receptors encoded by hundreds of genes in plant genomes. They function as a switch that determines when the plant immune system is activated. When a receptor gene in the plant matches molecules coded by a gene in the pathogen, the plant immune system is activated and blocks the infection. This switch is a fundamental molecular event that underpins the plant immune circuitry. The resulting immune response is complex and multifaceted but one hallmark is a localized programmed cell death reaction known as the hypersensitive response (Figure 1A). Nearly all plant cells have the cellular "suicide" machinery to restrict the spread of pathogen from the infection site to the neighbouring cells. This way plant cells can resist all types of pathogens and parasites, including viruses, bacteria, fungi, oomycetes, and nematodes.

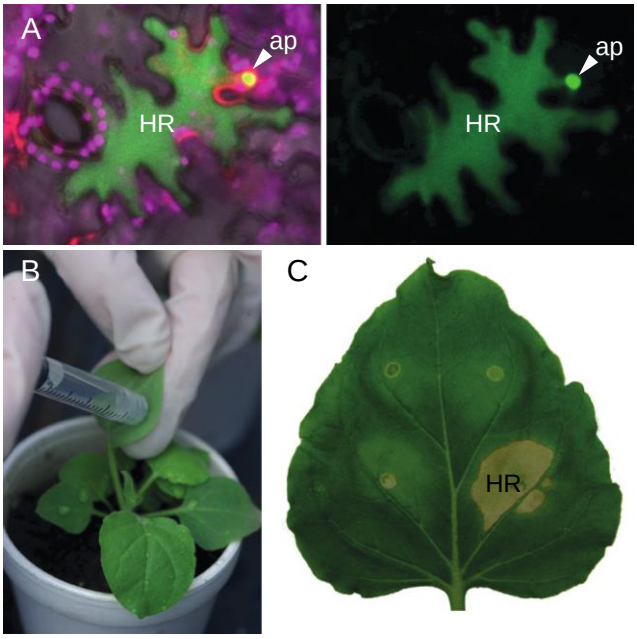

Figure 1. Hypersensitive cell death associated with plant immunity. A. Hypersensitive cell death in a single plant cell under confocal microscope. Plant epidermal cell showing cell death triggered by the potato blight pathogen *Phytophthora infestans* (ap) as colored in green. Chloroplasts are shown in magenta. B. Agroinfiltration method based on injection of *Agrobacterium tumefaciens* expressing plant and pathogen genes in the leaf of the model plant *Nicotiana benthamiana*. C. Image of hypersensitive cell death in *N. benthamiana* leaf after *A. tumefaciens* infiltration. HR: hypersensitive cell death.

The science of plant-pathogen interactions is known as plant pathology. Plant pathologists study all sorts of plants and pathogen, but they can experimentally reconstruct the hypersensitive cell death by artificially co-expressing plant receptor and pathogen ligand genes in a model system (Figure 1B-C). This type of experimental reconstruction of the connectivity between pathogen and plant genes has advanced our understanding of the mechanisms that underpin plant-pathogen interactions. Here, we review some of the key concepts that have emerged from the study of plant immunity.

Plant immune receptors

Plant immune receptors are classed into cell surface receptors known as pattern recognition receptors (PRRs) and intracellular receptors of the nucleotide-binding domain and leucine-rich repeat–containing (NLR) family. These two types of immune receptors are not restricted to plants but also occur in animals and a variety of other organisms. PRRs and NLRs detect pathogens and activate immunity through distinct but at times overlapping mechanisms.

How do plants detect pathogens?

Throughout evolution, plant immune receptors have acquired different mechanisms for detecting pathogen infections (Figure 2). In some cases, the receptor protein directly binds a pathogen molecule. In other cases, the receptor detects the cellular perturbations caused by the pathogen in the same way as a rodent infestation is generally detected through droppings or chewed up holes in a cereal box rather than by direct sighting of the pest. In this indirect recognition model, the receptor is viewed as guarding the cellular components that are targeted by pathogen virulence proteins known as effectors. In other cases, the host targets of the effectors became decoys—specialized host factors that bait the pathogen to function as co-factors of the immune receptor. More recently, another model has emerged in which the effector target has integrated as a novel domain within the receptor protein itself to directly bait pathogen effectors. These "integrated decoys" derive from host proteins associated with dozens of cellular functions, including signaling and secretory pathways. In many cases, the receptor carries only a fragment of the original host protein and thus has probably lost its ancestral function becoming a dedicated pathogen bait. The "integrated decoy" model has revealed that the host cellular pathways targeted by plant pathogens are extraordinarily diverse. Yet in many cases, plants have evolved to turn the table and trick the pathogen by baiting their virulence effectors in an endless evolutionary game of tic tac toe.

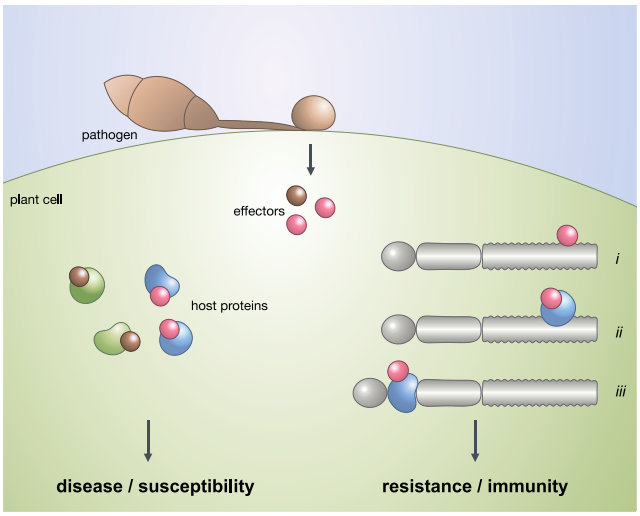

Figure 2. How to trick a plant pathogen. Plant immune receptors can detect pathogen effectors via three mechanisms as illustrated in the right panel: (i) direct binding of pathogen effectors; (ii) indirectly via a guardee/decoy host protein that is targeted by the effector; (iii) through an integrated decoy domain that originates from a host target of the pathogen. In the absence of matching immune receptors, the pathogen causes disease via the activities of its effectors that bind and manipulate host proteins (left panel).

How do plant immune receptors activate immunity?

PRRs and NLRs activate immunity through different mechanisms. PRRs are transmembrane proteins that carry an extracellular ligand binding domain and, in some cases, an intracellular kinase domain. The main function of PRRs is to sense pathogen-derived molecules in the apoplastic area—one of the first interfaces where plant cells meet pathogens and pests. A classic PRR is the Arabidopsis FLS2 protein, which binds and detects peptides derived from bacterial flagellin as non-self signals of immunity. In some PRRs, the ligand binding domain has evolved to detect damage-associated molecules derived from host plant cells. When plant cells face invasion, damaged cells fire up cellular components to the apoplastic area as a danger signal, loudly announcing the presence of an enemy to the surrounding cells. Upon ligand perception, PRR proteins, together with their co-receptor partners, activate immune signaling through phosphorylation and activation of downstream kinases such as the so-called receptor-like cytoplasmic kinases (RLCKs). PRR-mediated immunity is tightly correlated with this protein kinase-mediated phosphorylation cascade. It ultimately leads to dynamic physiological changes, including massive transcriptional reprogramming, deposition of callose at the cell wall, generation of reactive oxygen species (ROS) and, in some cases, activation of hypersensitive cell death.

NLR proteins are intracellular sensors that occur across all domains of life. In plants, NLRs are multidomain proteins that oligomerize into higher-order complexes upon direct or indirect activation by pathogen effectors. In the case of the Arabidopsis NLR protein ZAR1, pathogen detection occurs by binding RLCKs that are targeted by pathogen effector proteins in a process similar to the guard/decoy model described above (Figure 2). Activated ZAR1 oligomerizes into a wheel-like pentamer termed resistosome. It undergoes a conformational switch to expose a funnel-shaped structure formed by the N-terminal α helices. The resistosome complex is thought to mediate hypersensitive cell death by translocating into the plasma membrane and perturbing membrane integrity similar to pore-forming toxins. The membrane perturbations may consequently trigger other physiological responses associated with NLR-mediated immunity, such as ROS accumulation and immune-related transcriptional reprogramming. Whether all NLRs function like ZAR1 is not yet clear. However, although it is likely that a fraction of NLRs trigger cell death by direct membrane perturbation, NLRs appear to have evolved and diversified into multiple functional categories.

How did plant immune receptors evolve?

We are starting to have a clear picture of how plant immune receptors have evolved. The emerging model is that immune receptors originated from multifunctional ancestral receptors that have evolved into functionally specialized receptors that co-operate to form pairs and networks (Figure 3). Functionally specialized immune receptors can be either sensors dedicated to pathogen detection or helpers that trigger the immune response. Biochemical signatures of this asymmetrical evolution are prevalent in plant immune receptors. For example, the NLR sensors that have acquired extraneous integrated decoys typically cannot activate immune signaling on their own, but are dependent instead on co-receptors, helper NLRs. The outcome of this asymmetrical evolution is that sub-functionalized immune receptors became interconnected into networks of varying complexity. One such network, the NRC network, has expanded about 100 million years ago to comprise up to one half of the NLRs of tomato, potato and related plant species. This bow-tie network is defined by a large number of sensor NLRs that depend on a smaller number of helper NLRs to execute the cell death immune response.

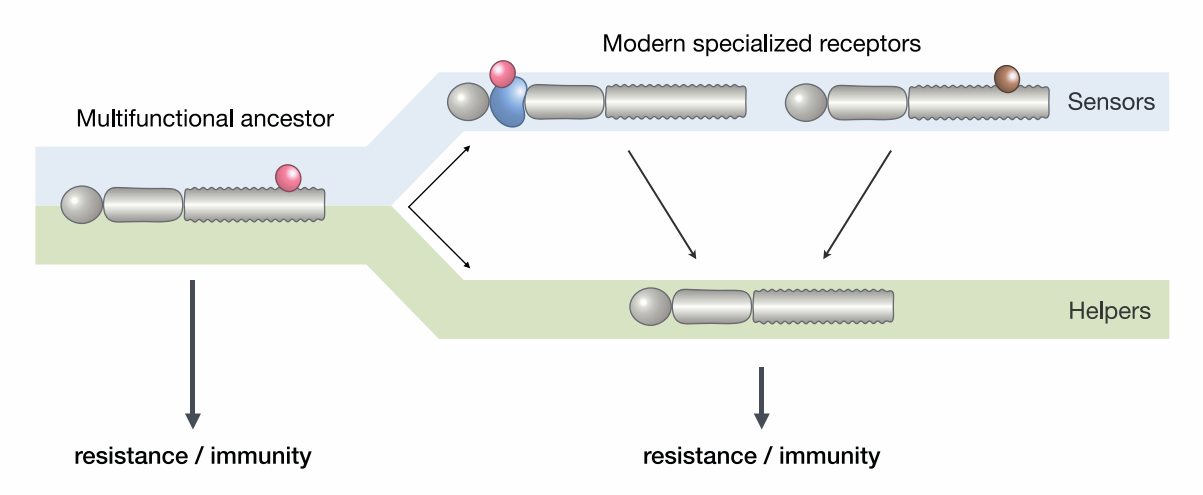

Figure 3. Asymmetrical evolution of plant immune receptors. Modern immune receptors generally originate from multifunctional ancestral receptors. Some of them have specialized to function either as sensors that detect pathogen effectors or helpers that trigger immune responses. As a result of coevolution with pathogens, sensors tend to evolve faster—they develop novel recognition specifies through de novo mutations or by acquiring new domains. They typically lose the ability to activate immune signalling on their own and instead rely on helpers to do so.

The network architecture of immune receptor circuitry confers advantages to plants and make them more robust to external perturbations. Networks provide redundant backup pathways that enable plants to effectively modulate their response to environmental stress. Also, functionally specialized receptors that operate as networks enable plants to evolve faster to keep up with the fickle pathogens that are continuously changing to evade the plant's immune system. For example, uncoupling pathogen detection from activation of immune signalling freed up sensor NLRs to integrate novel domains to bait the pathogen (Figure 3). Overall, the network feature of the immune system has enabled plants to keep up with their biotic enemies and probably contributed to the remarkable diversification and evolutionary success of plants.

How can basic understanding of plant immunity benefit agriculture?

In an age of rapidly expanding world population, we continue to lose too much food to disease. Understanding how immune receptors function and evolve sets the stage for their optimal use and deployment in agriculture. Some approaches focus on improving the utilisation of natural disease resistance occurring in the germplasm of wild crop relatives, whereas others aim at designing synthetic disease resistance genes with beneficial features.

The basic knowledge gained in the last years has direct implications for breeding plants for disease resistance. The effectiveness of the plant immune system rests on its capacity to detect invading pathogens. We are now in a position to decode the alphabet soup of plant disease resistance genes, which typically code for immune receptors, into functional categories and deduce the network topology of the plant immune receptors. This can guide strategies for retooling the immune system of crop plants for enhanced disease resistance, for example by boosting the expression of co-receptors to intensify the immune response mediated by multiple receptors, buffering negative network regulation by endogenous components and pathogens, and effective transfer of individual receptor genes across germplasm.

There are also strategies for improving plant immune receptors by expanding their pathogen detection spectrum (Figure 4). This can be done through introduction of mutations that expand pathogen effector binding or generate sensitized "trigger-happy" receptors with lower thresholds of sensor activation. Another strategy for engineering synthetic immune receptors is through domain engineering, either by manipulating naturally integrated domains or by introducing totally novel domains to recapitulate evolution. Although these approaches are still generally at the experimental stage, they promise to bring forward radically novel approaches to breeding disease resistance in crop plants.

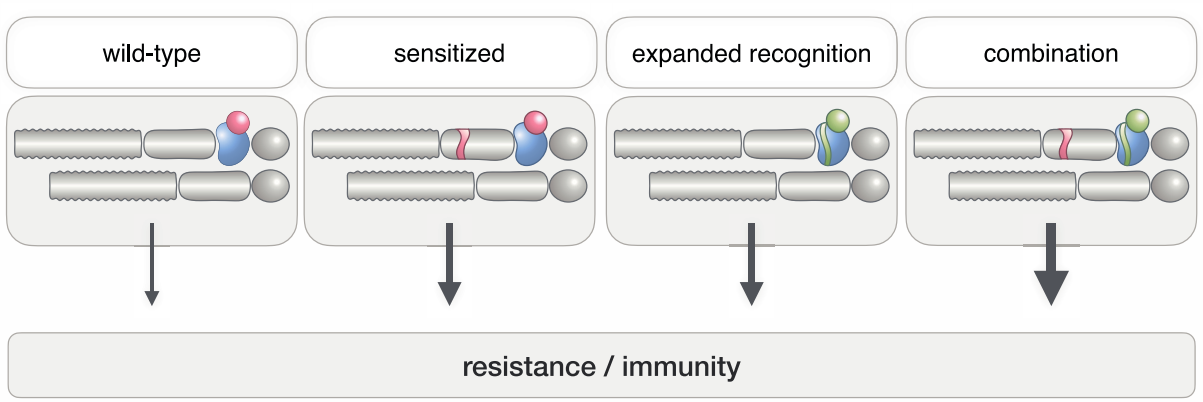

Figure 4. Strategies for improving immune receptors. Naturally occurring (wild-type) immune receptors can be improved by introducing "sensitizing" mutations that lower threshold of activation or by domain engineering to expand pathogen detection spectrum or switch recognition specificity.

Further Reading and Viewing

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