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Plant Immunity and PRRs

A GUIDE AND INTRODUCTION TO PLANT IMMUNITY AND PRRS

IGEM UZURICH, 2020

PRR Guide

When speaking of immunity and immune systems, we tend to forget that us mammals are not the only ones, who have an immune system. Plants have an immune system too! This guide aims to give an introduction and an overview of plant immunity. It is meant to enable people/laymen to grasp the basic concepts and ideas of plant immunity. Firstly, we will elaborate on what immunity actually implies, what an immune system is and how the immune system of plants compares to the one found in mammals. We will cover the principle idea of how an infection can arise and why plant immunity is of great interest, both, from a societal and economic point of view. It includes an overview of the two distinct branches of plant immunity and elaborates on the associated components. Lastly, the possible applications will be discussed, as well as how synthetic biology fits into the picture. As the title states, this guide aims to give a guide to plant immunity in general, whereby pattern recognition receptors (PRRs) will be analyzed in more depth.

Abstract/Introduction

Not only do mammals possess an immune system, but plants do as well. The immune system of plants has similarities with the innate immune system found in mammals. In plants, as in mammals, signaling has to occur, in order for an immune response to be triggered. Plant immunity is central for food security, one of the most important issues we have to face in the upcoming years. Without stable and efficient plant immunity, crop losses are expected, which again can have a huge social and economic impact. For a pathogen to successfully infect a host plant, three conditions have to be met, summarized in the so-called disease triangle. The pathogen must be able to overcome the host plants defense, the host must be susceptible to the pathogen and lastly, the environment has to favor conditions of which the pathogen will benefit. The plant's immunity can be divided into two general categories: Cell-surface receptor mediated immunity and cell-intrinsic immunity. The former is based on PRRs, membrane bound receptors, recognizing pathogen associated microbial pattern (PAMPs) in the apoplast. Pathogens have and will further evolve so-called effectors, which are aimed at inhibiting the initiation of an immune response through the activation of PRRs. A second line of defense is formed through NLRs, located in the cytoplasm. The immune response ensued after nucleotide binding-leucine-rich repeat (NLR) activation is considered to be much more volatile than if PRRs are activated. Both, PRRs and NLRs have distinct structural and functional components. Such receptor-proteins can be transferred between plants, allowing for engineering immunity. Traditional, molecular methods have already been implemented. In recent years, with a multi-scale, interdisciplinary approach in the form of synthetic biology, whole new pathways can be engineered, introduced and exchanged between the kingdoms of life. This will in return give deeper insight into plant development and signaling and harbors a huge potential for improving crop resistance. Through more in depth knowledge and the usage of novel engineering methods, the possibility to unleash the full potential of plants for the benefit on our health and environment arises.

Background: What is an Immune System?

Definition of an immune system

An immune system is a complex network of different cellular actions and signals, allowing an organism to defend itself against a pathogen. Simply put, as this is the case with most biological systems, the immune system is based on an exchange of input and output. In order for an immune response to occur, there must be an input. However, the immune system does this in a very unique way. If we think of the nervous system for example and look at a patellar reflex[1], we get an initial idea of how such and input-output system works. While testing the patellar reflex, there is a quick application of force (input) to the patellar tendon resulting in an extension of the leg (output). Anyone having experienced this reflex, knows how fast it occurs. Immunity, as a system, differs in this respect as it is generally operating at a much lower speed. Moreover, while the nervous system conducts information as an electrical signal, the immune system transmits information by chemical signals, or through the migration of cells. The immune system thus recognizes and protects the organism against pathogens, whereby foreign structures and molecules get recognized and an appropriate immune response is produced.

Innate vs. Adaptive Immune System

Important to mention is that in some cases the immune system can be divided into two categories, which are not mutually exclusive: The innate and the adaptive immune system. These two differ mainly in that, on the one hand, the innate immune system is inherited, while the adaptive one is acquired. Moreover, the innate immune system refers to nonspecific defense mechanisms that come into play immediately after a pathogen is recognized. The adaptive immune system refers to antigen-specific immune responses and is considered to be more complex than the innate one. Once an antigen of a pathogen is recognized, an army of cells is created, which will then recognize the specific antigen and attack it. Furthermore, the adaptive immune response includes a “memory process”, by which the immune system is trained to handle future infections with the same antigen faster and better.

[1] The reflex a doctor is testing when tapping below the kneecap and the leg consequentially (should) extend.

Plants versus Mammals

We as humans, or in more general terms, we as mammals are not the only species on earth, which possesses an immune system. Interestingly, plants do so as well. The immune system of plants shows several similarities with the immune system of mammals, differs nonetheless in some key aspects. In the following tables, the similarities and differences between the two immune systems are highlighted (1).

Differences	
Plants	*Mammals*
Innate Immunity: Resistance is inherited.	Next to the innate immunity there is also an adaptive immunity; the latter is not inheritable. Each individual must be infected to build up an immunity.
Each living cell is able to formulate a resistance reaction, as there are no mobile cells.	The immune reaction is formulated by specialized, mobile cells.
Receptors only recognize distinct structures (Effectors, MAMPs[2]). Furthermore, Effectors will mostly be recognized indirectly, often also through their function and not only through their structure.	Antibodies of the adaptive immune response are able to recognize almost any structure. However, mostly exposed surface structures get recognized.
The diversity of the receptors on the level of the individual is small, on the level of the population it is medium to high.	The diversity of receptors on the level of the individual and on the level of the population are both high.

Similarities
Innate immunity plays an essential role in defense response.
Initiation of the programmed cell death (apoptosis) after a successful infection of a pathogen.
Plants and mammals are equipped with receptors and are able to recognize MAMPs.
Receptors have similar structural elements such as LRRs[3], NBS[4]/NACHT[5] domains, kinase domains, TIR-domains.

[2] Microbial associated molecular pattern, MAMP

[3] Leucine rich repeat; LRR

[4] Nucleotide binding site; NBS

[5] NAIP (neuronal apoptosis inhibitor protein), C2TA (MHC class 2 transcription activator), HET (incompatibility locus protein from Podospora anserina) and TP1 (telomerase-associated protein); NACHT

Plant signaling

Since plants are sessile and do not possess any mobile cells, each cell has to be able to initiate an immune reaction, independent of other cells. Nonetheless, not only is there an intracellular flow of information (meaning within a cell), but there is also an exchange of information between cells. These processes are summarized under the term plant signaling. Plant signaling is defined as conveying information within and between plant cells form receptors systems to effectors. Signals are of chemical (and/or in some cases of electrical) nature and can occur locally within a single plant or even between different plants, including plants of different species (2). These signals will trigger complex networks of interactions to orchestrate biochemical and physiological responses. Examples for such responses are flowering, fruit ripening, germination or, what we are interested in, an immune response. Oftentimes, such signals will be recognized by a receptor and are followed by a transmission of said information to the nucleus via a complex network (3).

Importance of plant immunity

Producers, Consumers and Decomposers

Before we do, however, look at how plant immunity functions and analyze the components in more detail, we should first answer the question of why we are interested in studying plant immunity in the first place. What relevance does plant immunity have for us humans and how important is it really? Humans are dependent on other species to obtain the energy necessary for our survival. In general, we can classify us as being consumers. Consumers are one of three distinct categories, by which we can characterize all living organisms. The other two categories are producers and decomposers. Producers are generally photosynthetic organisms that will generate energy by making use of sunlight. Certain organisms have also found a way to produce energy rich compounds in light scarcity or even in the complete absence of light. These are called chemotrophs (4). Consumers, on the other hand, are not able to produce energy on their own and will depend on the consumption and digestion of producers and/or other consumers to do so (5). Decomposers will, as the name suggest, decompose and break down organic material, such as the remains of a dead organism. Decomposition is an important process, as it allows for organic material to be recycled and reintroduced into an ecosystem (6). Taken all together, producers, consumers and decomposers will allow for a flow of energy/ organic material, forming a food chain.

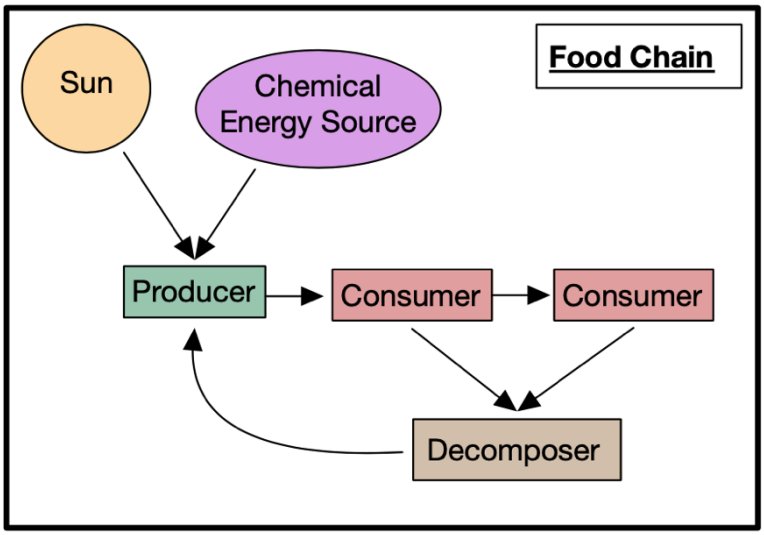

Fig.1. Food Chain
Diagram depicting the interaction between producers, consumers and decomposers

Humans and plant immunity

Circling back to our initial statement that humans are consumers and not producers, it should become evident, how we depend on plants in the most fundamental way possible: We are dependent on plant material for energy. Wheat for example, with almost 750 metric megatons being produced on an annual basis, is estimated to cover ~15% of our daily, globally consumed calories alone (7). Wheat can also be targeted by pathogens, leading to crop loss. It has been estimated that more than five million tons of wheat production are lost annually to yellow rust, a wheat pathogen, alone (8). Overall, it is believed that the annual crop loss of 20-40% occurs, due to pathogen infections. From this observation alone, it is evident, that the ability of a host plant to effectively defend itself against pathogens, has a direct impact on food security. Furthermore, crop losses can also have a direct impact on both the national economy of a country and the household livelihoods (9) (10). An example to underline the importance, relevance and implications of plant immunity even further is the great famine during the years of 1845-49. This period is often also referred to as the "Irish Potato Famine", a period in Ireland, during which huge potato crop losses occurred. The crop losses were caused by an infection of *Phytophthora infestans*, more commonly known as late blight. Not only did 1 million people ($\frac{1}{8}$ of the total Irish population) die of starvation or famine-related illnesses, but moreover a large amount of people was driven to emigrate due to the vast food shortage. Later, during World War I, it is thought that late blight had damaged German crops and therefore helped to end the war (11).

Disease triangle

Pathogens can also be classified as consumers; therefore, they also have a dependency on plants to produce energy. The plant will naturally try to avoid this. Hence, plants and pathogens are in a constant battle. The pathogen wants to infect the host, the host will try its best to defend itself against the attack. If we consider that roughly 25% of the plant genome are dedicated to respond to pathogen infection, it becomes evident, that infection and disease actually are not the rule, but rather the exception. Most plants are resistant to most pathogens. Plants are exposed to a myriad of different pathogens on a daily basis, but only a select few are able to successfully infect them. Why is this and what makes an interaction between a plant, and a pathogen into a disease? To answer this question, we will look at the so-called disease triangle.

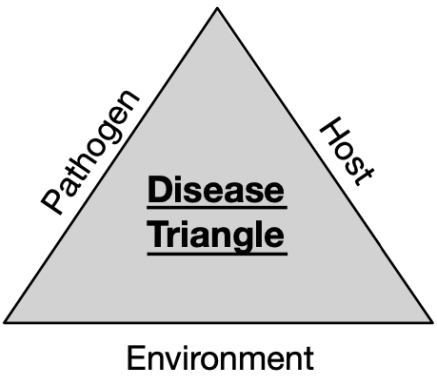

Fig. 2. Disease Triangle
Factors influencing the success chance of a pathogen infecting a host cell/organism and how they are connected to one another.

Each axis of the triangle depicts one criterion which must be fulfilled for an infection to take place. The first axis is dedicated to the pathogen itself. The pathogen must be able to overcome the plant's defenses. The second axis is the axis of the host, indicating that the host plant must be susceptible to the pathogen. The third and last axis is dedicated to the environment. Not only does infection depend on the pathogen and host, but depending on the environment, the balance can be tipped either in favor of the plant or in favor of the pathogen. If the environment strongly favors the plant, no infection will occur, even though the plant would be susceptible to it, and the pathogen would be able to overcome the plants defenses. An interaction between host plant and pathogen will be characterized as a disease, if the pathogen manages to find and attach itself to the correct host, gain entry through the plant's defenses and avoid its defense responses. In order for it to be able to spread to the next cell or organism, it must further be able to grow and reproduce with its host and then be able to spread. A pathogen has diverse options of spreading. Wind, water and insects can help a pathogen to reach their next host (12).

An Overview of Plant immunity

From a conceptual point of view plant immunity can be divided into cell-surface immunity and intracellular immunity. The cell-surface immunity can be seen as a first line of defense. If a pathogen is in close proximity of the plant cell and a signature is recognized, an immune response will be elicited. If the pathogen overcomes this first-line of defense and manages to penetrate a cell, the intracellular immunity comes to play. While the cell-surface immunity is achieved via cell-surface receptors, whereby pathogen signatures are recognized by the extracellular domains (ECDs), activation of the intracellular immunity is triggered, if pathogen signatures are recognized inside of the cell. Hence, cell-surface immunity is mediated through pathogen-recognition receptors, referred to as PRRs. The cell's intrinsic immunity is mediated via NLRs (nucleotide-binding leucine-rich repeat immune receptors). In other words, intracellular immunity receptors called NLRs detect signatures of better adapted pathogens. These signatures are translocated proteins, aimed at modulating the metabolism or the physiological response of the host, maximizing the pathogenicity. The translocated proteins are termed "effectors". Once an effector is detected, the ensuing immune response of the host is considered to be more robust, oftentimes being associated with localized cell death. If the targeted cell dies, the pathogen cannot grow and replicate, thus breaking the infection cycle (13).

Effectors

Before discussing the interaction between plant host and pathogen in a more detail manner and giving some molecular biology insight, the role of effectors must be determined. Effectors can be defined as molecules used by a diverse array of organisms for the regulation of the activity of another organism. Effectors do not exclusively have to originate from organisms we traditionally associate with pathogens, but rather can be derived from plants, animals and microbes alike. In this guide, the term "effectors" will refer to the proteins stemming from microbial pathogens, intended to increase the chance of infecting a host plant. In other words, effectors are aiming to suppress the plant's immune response and/or are contributing to the pathogen's viability. More specifically, effectors will try to inhibit the PAMP-triggered immune response (see below) and achieve this either by being secreted into the extracellular space or into the host cell. This ultimately means, that effectors act outside of the pathogen and in the cell or apoplast. There are many different targets for an effector to modulate. One example

would be the AvrPto from *Pseudomonas syringae*, which is targeted at interfering with the signaling of FLS2 (see below). AvrPtoB inactivates the kinase activity of BAK1 by masking its active site and substrate-binding surface, essentially blocking an interaction with FLS2 (14). Many effectors of bacterial origin are introduced into plant cells through the Type-III secretion system (T3SS). The T3SS is based on complex bacterial structures, enabling the bacteria to inject effectors into the cytoplasm, effectively bypassing the extracellular milieu (15). Nematodes introduce their effectors through a feeding stylet, while fungal and oomycete effectors are often secreted from haustoria or the tips of hyphae. However, effectors can be beneficial as well as deleterious for the pathogen. Since they are foreign to the plant and can be recognized as such, plant immune receptors both at the cell surface level and intracellular level are able to recognize such effectors. Unsurprisingly, there is a very high selective pressure associated with effectors. Effective pathogens show a very high rate of effector innovation. *Phytophthora infestans* for example has a very large genome, full of duplications and repetitive elements. *Phytophthora* effectors are encoded in transposon-rich regions, meaning that they are found in regions of their genome which are exposed to a very high rate of recombination and diversity. This will increase the chance of phytophthora producing an effective effector, allowing for a successful infectious cycle to take place. Figuratively speaking, effectors are the weapons of a pathogen and high effector innovation leads to an increase in pathogenicity (16) (17).

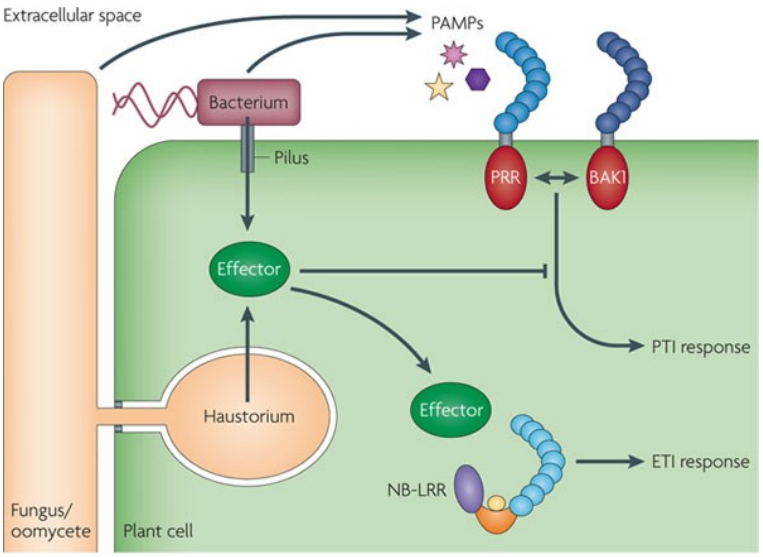

Fig. 3. The principles of plant immunity
Graphical representation of the underlying principles of plant immunity. Molecules associated with or which are secreted by a pathogen into the extracellular space (PAMPs), are recognized by cell surface immune receptors and trigger a PTI response. Effectors, oftentimes suppressing the PTI, will then be recognized intracellularly through NLR, triggering stronger immune response, referred to as effector trigger immune (ETI) response. Note: Effectors can also be secreted into the extracellular space (16).

Cell-surface immunity: PRRs

Structural and functional components & diversity

Pathogen recognition receptors or PRRs are localized at the plasma membrane and monitor the apoplast. The apoplast denotes the space between the cellular membrane and the cell wall of plants. It is formed through the continuum of adjacent cell walls and serves as an important route of transportation of a diverse array of solutes and water within the tissues of a plant. Structurally, PRRs have three distinct parts. The first one, found on the outside of the cell, is termed as the extracellular domain and is responsible for the signal recognition. Ligands to this domain, meaning pathogen signatures binding to this domain, are defined as MAMPs (microbial-associated molecular patterns, or DAMPS (damage-associated molecular

patterns. PRRs are anchored into the plasma membrane through the transmembrane domain. As the name suggests, this region of the PRR spans the region, which is integrated into the plasma membrane. On the intracellular side of the cell surface immunity receptors a kinase domain is either present or absent. The kinase domain is thought to be responsible to signal transduction. If a kinase domain is present, the PRR will be classified as a RK (receptor kinase). If no kinase domain is present, the PRR will be classified as a RLP (receptor like protein). Both RKs and RLPs are considered to be PRRs. In *Fig.3.* a graphic of different RKs and RLPs is shown.

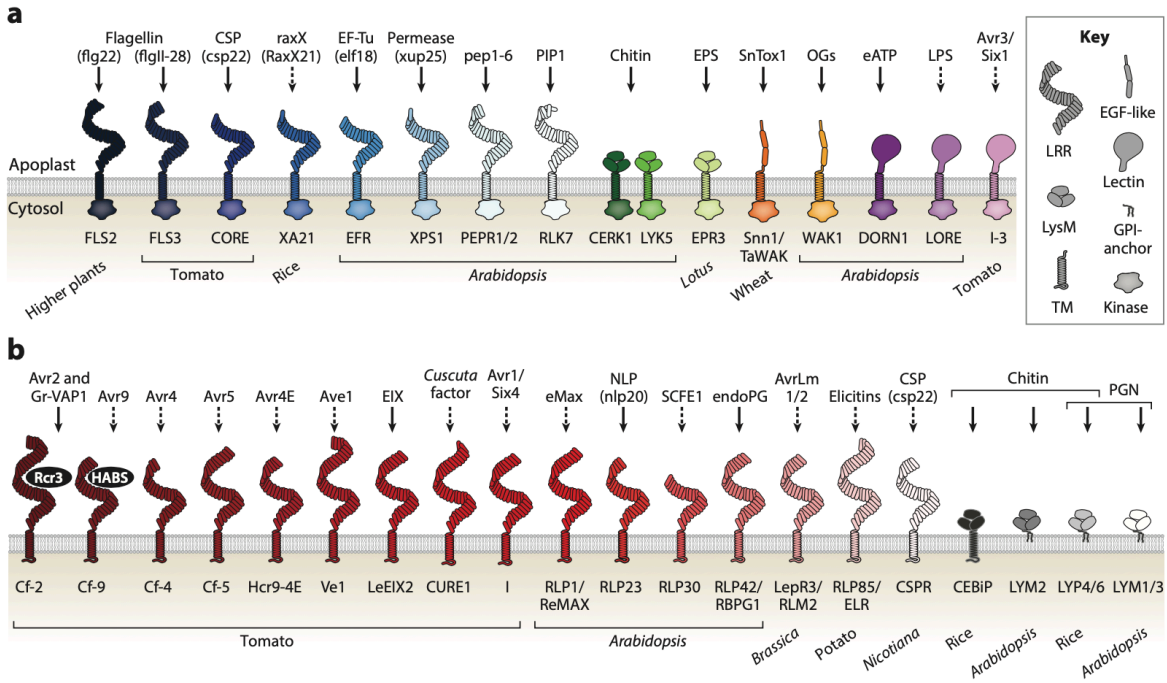

Fig. 4. PRRs with Ligands
(A)Receptor Kinases, (B)Receptor-like Kinases (RLPS) with their respective ligands and in which organisms they have been found. Structural domains of the RKs and RLPs are depicted in the legend in the top right corner (17).

It has been found that RLKs not only regulate immunity through the recognition of non-self signatures in the apoplast, but further are involved in regulating the response of abiotic stressors, reproduction as well as growth and development. It is not uncommon, that these surface receptors are in need of a co-receptor to transduce perception of PAMPs/DAMPs. PAMPS, which are recognized by the cell surface immune receptors, are usually essential to the fitness of the pathogen and therefore tend to be evolutionary constrained. PAMPs can include structural elements of the cell wall or bacterial flagellin. Because these structural elements tend to be evolutionary constrained and can be found in several different types of pathogens, the immunity achieved via PRRs is considered to be of a broad-spectrum. As for an example, with one PRR, several different bacteria can be recognized (13) (18).

Sub-Families of RKs and RLPs

As mentioned before RKs and RLPs both consist of an extracellular domain, a transmembrane domain and differ only in the presence or absence of an intracellularly located kinase domain. Based on the type of ECD present, RLPs and RKs can be clustered into distinct sub-families. Examples for such sub-families are the leucine-rich repeat (LRR), lysine motif (LysM), lectin, and epidermal growth factor (EGF) domain-containing receptors. Depending on what ECD is present, the nature of the perceived ligand changes accordingly. The different ECD and the diversity of ligands binding to the respective receptors are visualized in Fig. 4. Some of the most well understood PRRs are the RKs FLS2, EFR and the LysM-type RKS LYK5 (Lysin motif receptor kinase 5) and CERK1 (Chitin elicitor receptor kinase 1) (13) (18).

FLS2, flg22, BAK1 and BIR1

In order for the reader to obtain a more in depth understanding of how the signal recognition at a cell-surface receptor functions and how the following intracellular signaling cascade works, we will discuss the proposed model of how FLS2 operates. FLS2 is a good PRR to study, as some variant of it is found in most plants. But first, how does FLS2 structurally look like? FLS2 can be clustered with RKs, as it contains an intracellular kinase domain. On the extracellular side we find a LRR domain, which is linked to the intracellular kinase domain through a helical, single pass, transmembrane domain. One single repeat of the LRR domain consists of ~24 amino acids, whereby a consensus sequence has been proposed (LxxLxxLxxNxLxGxIPxxLGx). This sequence will then be repeated 28 times, forming the functionally active LRR domain. On a side note, not all LRRs are alike, as the LRR of EFR consists of only 21 repeats. This makes sense, if one considers that these two receptors are targeted against different signatures of pathogens. Now that we have established the structural side of FLS2, how does it function? In the inactive state it is thought that FLS2 is present, bound to other inactive FLS2 receptors, forming dimers or oligomers. As elucidated previously, some cell surface receptors need a co-receptor for their activation to occur. In the case of FLS2 it is BAK1, which is needed. BAK1 also possesses an LRR domain for its ECD, which is linked to a transmembrane domain, with a Serine/Threonine kinase like domain on the intracellular sides (19). At this intracellular kinase domain of both, FLS2 and BAK1, we find BIK1 attached. Hence, BIK1 associates with FLS2 and BAK1 kinase domains. BAK1 is thought to form a complex at the membrane with BIR2. If a PAMP, in this case flg22 of bacterial flagellin, binds to the FLS2 LRR domain, the di- or oligomeric complex dissociates. More specifically flg22 will bind to the FLS2-ECD via the LRR subunits LRR3-LRR16. This mainly happens through the formation of hydrogen bonding, electrostatic and hydrophobic contacts. FLS2 now forms a complex with its coreceptor BAK1, meaning that the BAK1-BIR2 complex will dissociate too, if a signal is recognized. Further, at the intracellular kinase domains of FLS2 and BAK1, BIK1 will dissociate as well, allowing for these domains to become active. FLS2 and BAK1 will dimerize, resulting in the phosphorylation of the respective kinase domains. The flg22 acts as a sort of glue, stabilizing the heterodimer. This will trigger a whole signaling cascade, aimed at producing an appropriate immune response. Put differently, BIR2 is a substrate for BAK1, which will be released upon PAMP perception, allowing for the FLS2-BAK1 dimer to form. Therefore, the FLS2-BAK1 complex formation is negatively regulated through BIR2 (13) (20). If triggered, the kinase domains will induce callose deposition, defense gene expression, stomatal closure, MAPK signaling and/or ROS (reactive oxygen species) bursts. Since the kinase domains initiate the signaling cascade intracellularly, it should become evident, how important the regulation of these domains through, for example BIK1, is. The PAMP-triggered immunity of cell-surface

receptors is considered to be less volatile than the intracellular one. As mentioned previously, the latter one will often result in localized cell death (13).

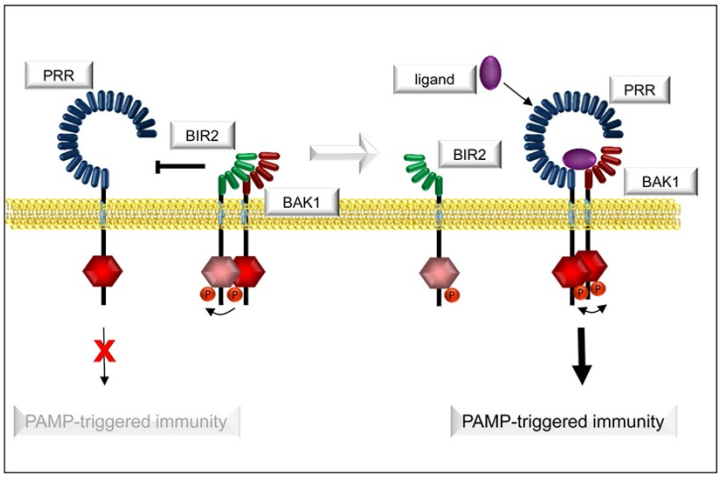

Fig. 5. PAMP triggered immunity of an LRR RK
Initiation of a PRR mediated immune response after signal recognition at the LRR domain of the PRR. Once the PRR (e.g. FLS2) and its co-receptor BAK1 form a complex, an immune response is initiated (20; 34).

In addition to what has been mentioned, it should be clarified, that this is a very simple and reduced overview of what is thought to occur at the membrane. It is thought, that there is a lot of additional complexity (posttranslational modifications, crosstalk between different receptors, etc.) present, which will not be covered here as this would go beyond what this guide is aiming at.

Intracellular Immunity: NLRs

Overview

As previously mentioned, PAMP-triggered immunity (PTI), originating from activated cell-surface receptors, is not the only way a host plant can defend itself against a pathogen. The intracellular immune response, mediated through NLRs, is a secondary option and is considered to be a much stronger immune response. These NLRs perceive the presence and/or activities of host translocated effectors. In other words, NLRs, are localized in the cytoplasm and detect injected pathogen effectors. Once activated, the heightened defense responses include the production of the stress hormone salicylic acid (SA), production of ROS, expression of pathogenesis-related (PR) proteins, the hypersensitive cell death response (HR) and lastly, the release of systemic signals resulting in systemic acquired resistance (SAR). Especially the HR response is of significance, as this is a very drastic strategy to limit the spread of the infection (16).

Structural and functional diversity.

Plants have variable numbers of NLRs encoded in their DNA, indicating extreme expansions and contractions of the genome. For example, *Triticum aestivum* (common wheat) has a total of ~1250 NLRs, while *Zea mays* (maize) has only roughly ~100 NLRs (21). NLRs are comprised of an LRR domain, a central nucleotide-binding domain (NB-ARC) and a variable module, typically being either a TIR (Toll/interleukin-1 receptor/resistance) or a coiled-coiled domain (22). As we have seen, the LRR domain is also present in cell surface receptors. It is thought that the LRR domain is implicated in effector recognition for some NLRs and further, in the autoinhibition of the receptor (23). The NB-ARC domain can be viewed as sort of a molecular switch (22).

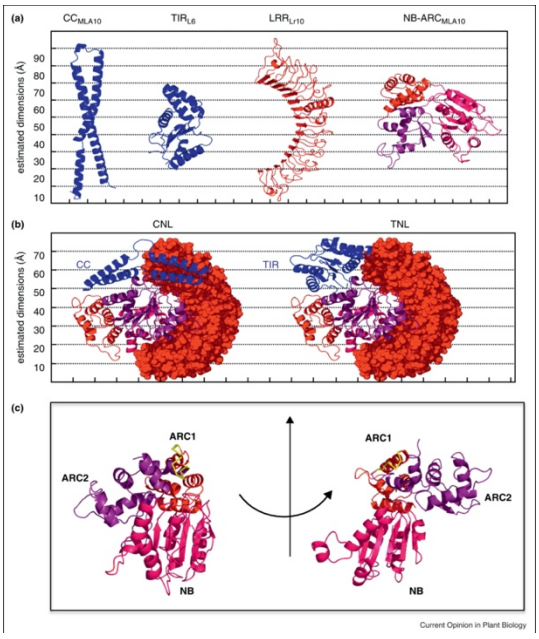

Fig. 5. Model of structural domains of NLRs
(A) Structural domains of the NLRs CNL and TNL. (B) Model for folding of CNL and TNL (C) Conformational change in the NB-ARC domain, which is thought to happen during activation (22).

Effector detection

Before looking at how exactly the NLR activation occurs, it would make sense to first establish, how effectors are detected in the cytoplasm. In principle there are three different ways in which it is assumed that NLRs detect effectors: the direct recognition model, the indirect recognition model and the integrated domain recognition model. The indirect recognition model can be further split up into guardee and decoy model (24).

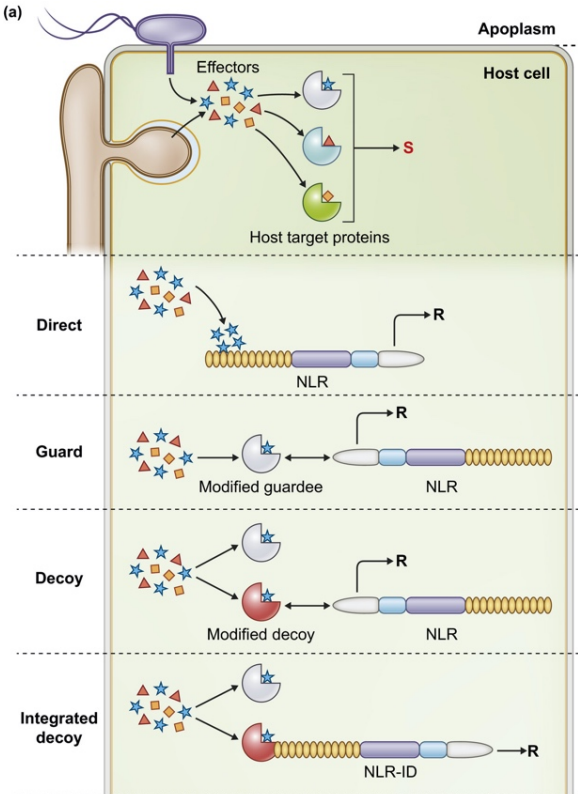

Fig. 6 Models for effector recognition via NLRs.
There are three different models, the direct, the indirect and the integrated domain recognition model. The indirect model can be further split up further into the guard model and the decoy model (24).

In the direct recognition model, it is assumed, that the effector molecule will interact directly with the LRR domain of an NLR. This model is best characterized in Flax, whereby it has been shown, that the polymorphisms in the LRR region can be associated with immune specificity (25) (26). In the indirect model the NLR is thought to surveil and monitor a certain guardee or decoy. If the guardee or decoy is changed in any way, the NLR will be activated and an immune response shortly ensues. In the guard model, the guardee serves an important role in the host cell, while in the decoy model, the guardee mimics the actual effector target, however, has no real other function outside of immunity (24). The integrated domain model is sort of a may be described as a combination of the first two: the NLR has a domain, serving as a sort of a sensor, by directly interacting with effectors. An example for such an integrated domain is the heavy metal-associated (HMA) domain found in rice receptor proteins Pik-1 and the Pia sensor NLR (RGA5). It has been shown, that HMA domains interact with the pathogen's effectors (27) (28).

NLR activation

If an effector is present in the cytoplasm and is detected by an NLR, a conformational change, usually in the form of domain rearrangements and oligomerization, occurs. More precisely, if an effector is perceived in the inactive state, the NLR will be activated through a conformational change, most probably in the NB-ARC domain where an exchange of a ADP for an ATP (nucleotide exchange) occurs (29).

Singletons, Helpers and Sensors

NLRs have evolved to function on their own, in pairs and as parts of a complex, interconnected network. Some NLRs have been identified and characterized to sense and elicit an immune response. These are referred to as singletons (30). However, the majority of NLRs are classified either as sensors or helpers. In pairs. The sensor NLRs perceive the effector, while the helper NLR will activate the immune response itself. The sensor NLR regulates the activity of the helper NLR. If we think of the immune response associated with NLR activation (HR), this becomes more intuitively clear. If the reaction is to introduce localized cell death, this mechanism has to be tightly regulated. With NLRs working in pairs, this is done more efficiently, than this is the case with singletons (31).

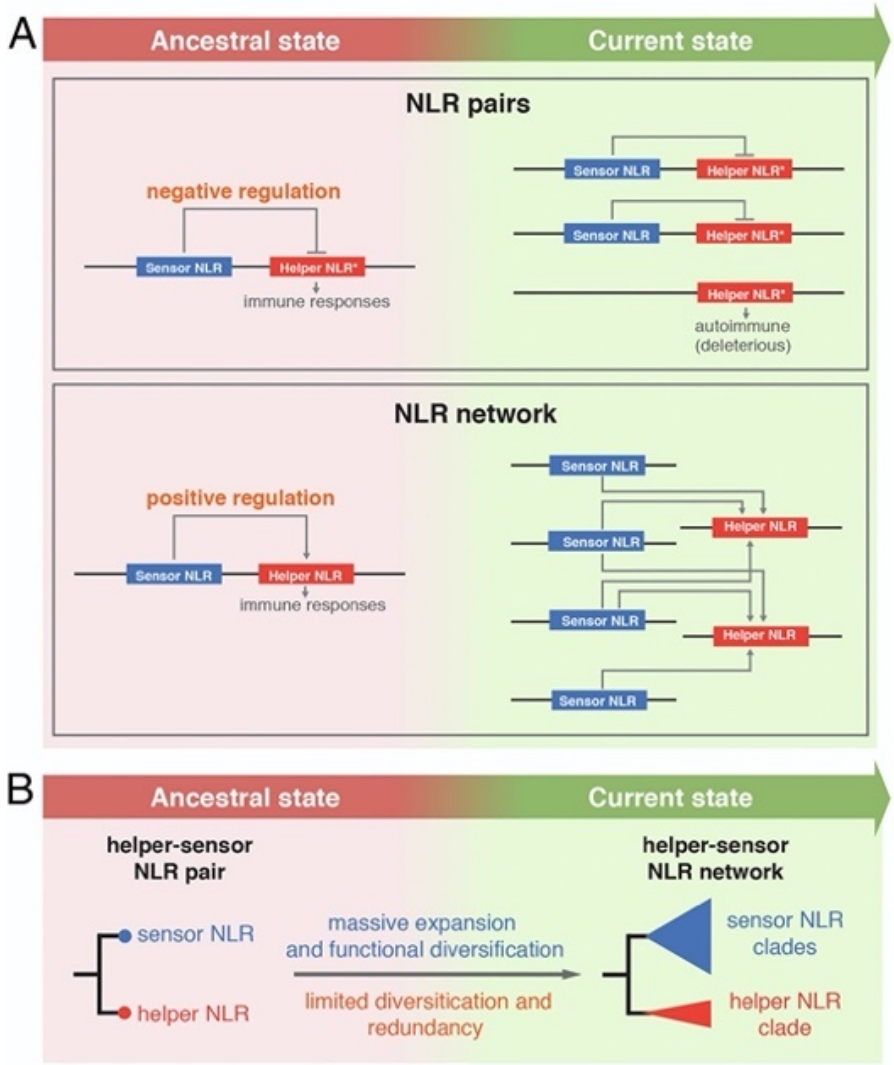

Fig. 7. NLRs functioning in pairs or in interconnected networks

(A) NLR pairs are comprised of sensor NLRs regulating the activity of helper NLRs. Moreover, NLRs can function in networks, whereby there are more sensor NLRs regulating the activity of helper NLRs.

(B) A massive expansion functional diversification of sensor NLRs and a limited diversification of helper NLRs allowed for networks to form. (31)

Engineering Plant Immunity

Transferring Genes and Boosting Signaling

Several biotechnological approaches have been aimed at improving disease resistance in plants especially crops. Such approaches include altering the amino acid sequence of receptors in order to improve the effector binding or modulate receptor activity or transferring resistance genes between species. As monocultures show a reduction in natural resistance diversity, crops have becoming increasingly vulnerable to pathogens. While the topic of GMOs is still heavily debated, editing genomes of crops, giving them resistances against different pathogens, might offer potential solutions for food security. While the transfer of cell-surface receptors will allow for a more broad-spectrum disease resistance in other plant species, the transfer of NLR genes will allow for a more targeted immunity against specific pathogens. Both have already been done (32). For example, transferring EFR, commonly only found in *Brassicaceae* species allowed for the engineering of a novel, broad-spectrum resistance in tomatoes and rice (33) (34). There are several other approaches to installing novel and/or improved disease resistance in plants. Next to the cell-surface receptor genes and R-genes (encoding for NLRs), the signaling pathways active during an immune response are also biotechnological targets. Early attempts aimed at overexpressing individual components of the immune response, such as the production of ROS, cell-wall fortification (cellulose deposition) or localized cell-death (35).

Synthetic Biology and Plant Immunity

Molecular genetics as we know it, has allowed for engineering plant immunity and making crop improvements as described above. However, recent technological and computer scientific advances resulted in an accumulation of an enormous amount of new data, uncovering the underlying principles even more, leading to a deeper understanding of plant physiology and immunity. Synthetic biology and new molecular engineering approaches build on these findings. Regulatory circuits composed of receptors, ligands and signal transduction components, as well as epigenetic machinery and molecular motors can be designed and introduced into plants, creating novel, desired traits. For example, there are reports on a ground-up design of a complete, artificial signaling pathway for detecting environmental pollutants, based on an engineered bacterial two-component system (36; 21). A two-component system usually consists of a membrane-bound receptor, acting as a sensor, and an intracellular response regulator. Upon activation, conformational changes are triggered, and target gene expression ensues (37). Further results imply, that two-component systems can be introduced or exchanged between the kingdom of life, legitimizing the potential repurposing of this module for diverse applications (38). Engineering receptors could be applied for manipulating plant growth and symbiotic relationships while limiting parasitism. In principle these approaches, and the associated possible implementation of new findings, are in part limited through our knowledge of the genotype-to-phenotype map. A deeper understanding thereof will allow for a more targeted approach and prototyping optimal candidates for engineering. Synthetic biology will only enhance our understanding of plant development and signaling and ultimately allow us to unleash the full potential of plants for the benefit of our health and the environment (39).

Closing Words

Plant immunity impacts our daily lives more than we know. In part, it has shaped the world as we know it today and is a key player in the future of our society. Consequentially, a deep and thorough understanding of plant immunity is needed. Not only should this be approached from the perspective of plants exclusively, but the pathogen and the effect of the environment must be considered as well. Although a wide array of different cell-surface receptors as well as cell-intrinsic receptors have been identified and linked to immunity, their exact biological role and ligands still remain an unsolved mystery. As we progressively understand more and more how these different parts work and where they are located in the overarching network/ building plan of the organism, opportunities arise, for engineering and improving such systems, in order to combat crop loss and global food security problems for example. However, we should not limit ourselves to the possible applications of such new findings. Plants form an immense reservoir of untapped resources, which, with the right thinking and approach, may be utilized to improve different areas of our life and our environment.

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