How to trick a plant pathogen

There's a bright future for bioengineering of plant immune receptors, a future that's deeply rooted in fundamental research and discovery.

This article was adapated from a talk presented by Sophien Kamoun at Translational Research in Crops, 22–23 June 2023, Ghent, Belgium. Click here for the video.

Preamble

All set to go? Fantastic. As the saying goes, I'm the only thing standing between you and the conference boat trip. I'll aim to wrap up on time, but given the rain, perhaps I can indulge in a little extra time.

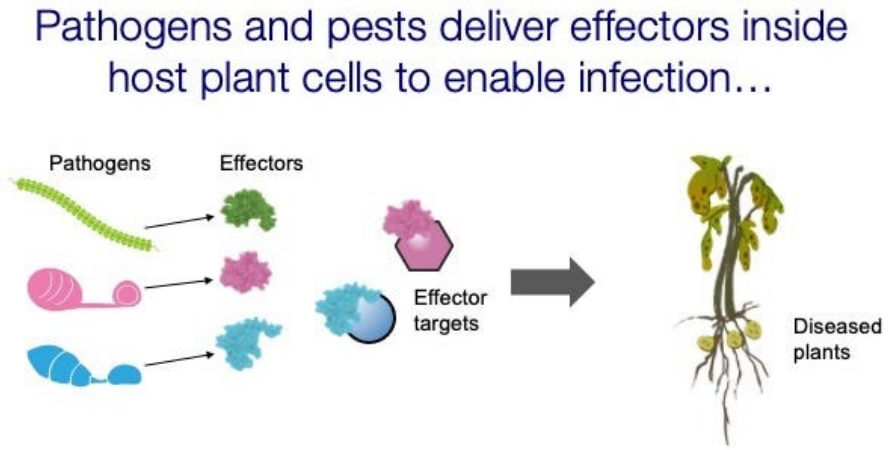
It didn't rain after all.

A brief introduction to the world of plant-pathogen conflict

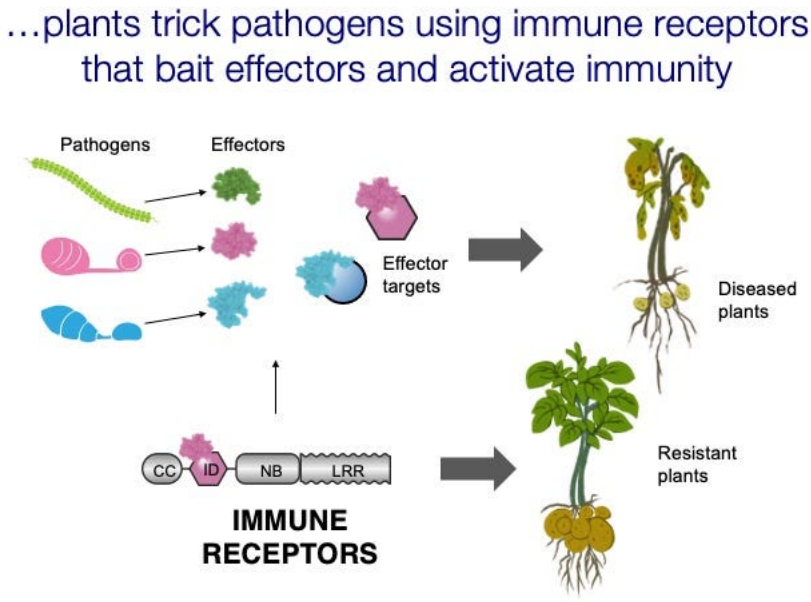
I'll continue on the theme of plant pathogens. Thanks, Barbara de Coninck, for illustrating the significance of plant pathogens. We lose substantial crops to pathogens. In fact, 2020 was the International Year of Plant Health, as declared by the UN. Of course, it coincided with a global pandemic that somewhat overshadowed the plant health cause. Nevertheless, we still lose a significant amount of food to diseases and pathogens.

Let's delve deeper into our field.

From a fundamental perspective, the field of plant-pathogen interactions has coalesced around some robust principles and concepts, which I will be illustrating. The first one is the fact that pathogens and pests, whether they are fungi, oomycetes, nematodes, aphids, and so on, all secrete effector molecules, usually proteins. These effectors have targets in the host; they modify plant processes, aiding the pathogen in causing disease and establishing themselves to colonize the host.

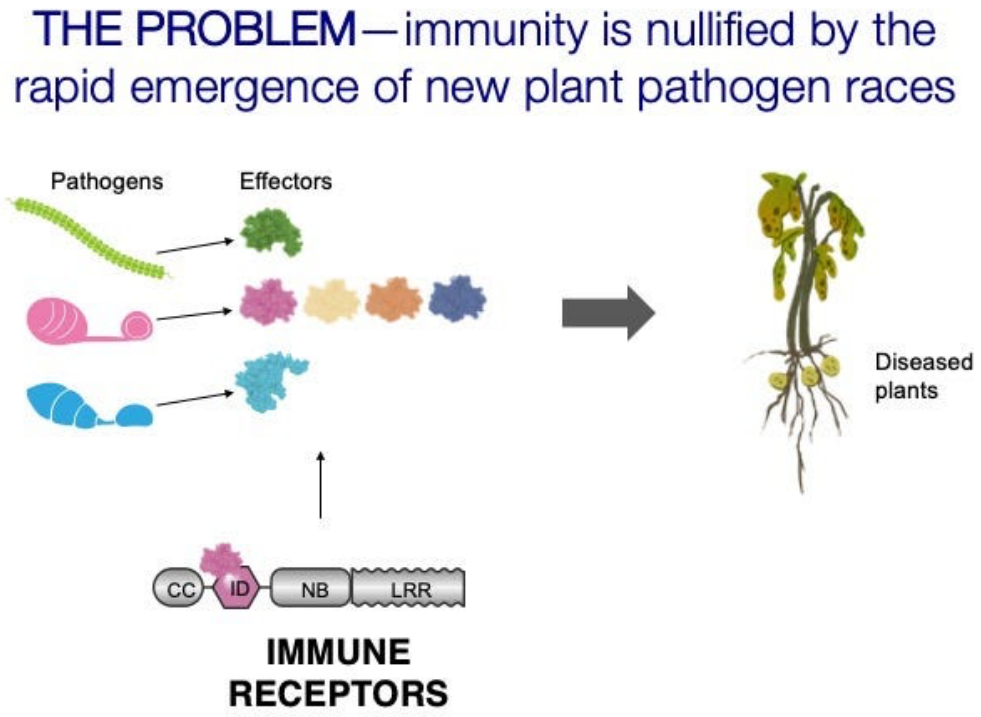


However, plants fight back. They possess highly effective immune systems. Their first lines of defense are the immune receptors that can detect and trick the pathogens. There are multiple mechanisms by which these receptors detect the pathogens. One innovative solution plants have come up with is to turn the tables on the pathogens and adopt the targets of the effectors as bait or decoys. This strategy effectively tricks the pathogens and aids in their detection.



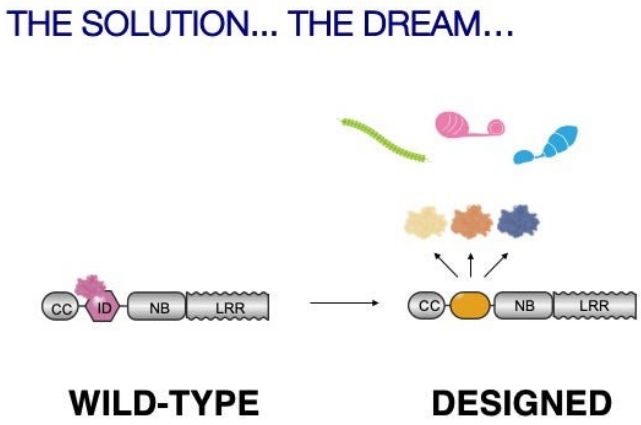
I will return to these examples later. If plants possess such robust immune systems with these receptors, what then is the issue? The issue lies in the fast-evolving nature of pathogens.

These pathogens are exceptionally adept at evading plant immunity, generating new races that can bypass the immune receptors. For instance, in this scenario, the pink effector has evolved into the yellow, orange, and blue variants. This type of evolution occurs constantly, especially in agricultural systems, where these pathogens are subjected to extreme selection pressures.



So the problem isn't that plants lack an immune system or immune receptors, but rather that the pathogens evolve exceptionally quickly.

The solution, or even the dream within our field of plant immunity, is the capacity to produce designer resistance genes or custom-made receptors. This is indeed the "holy grail" of our field: Can we take wild-type receptors and bioengineer them to create designer genes that can detect any pathogen we'd like?

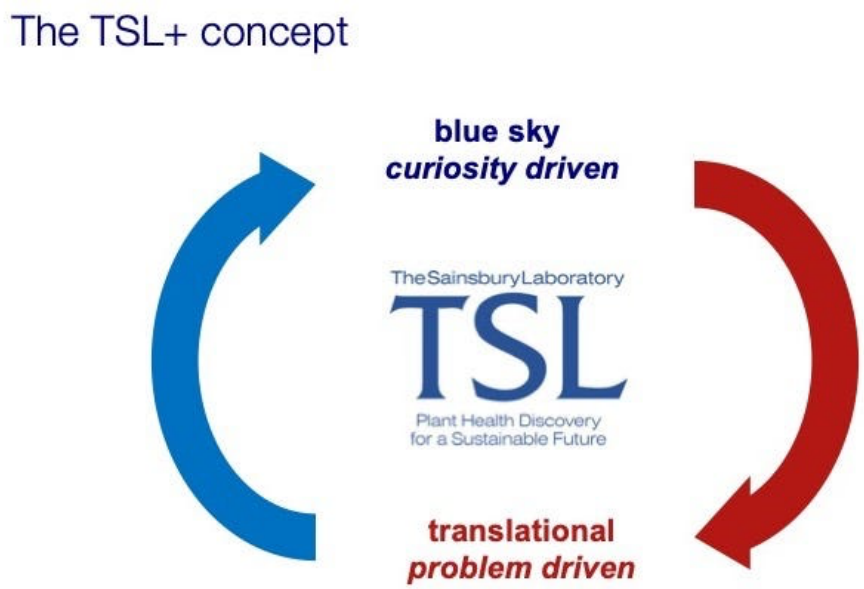


Gained in translation

This is the backdrop for my field. As the field has solidified around robust concepts, we at The Sainsbury Laboratory began to realise about a dozen years ago that we would be missing an opportunity if we didn't invest more in application and translational science.

This view aligns perfectly with the theme of this conference, because what we decided about 12 years ago was to start a program we called TSL+. This program expanded the lab beyond its original mission of curiosity-driven, blue-sky research, where investigations were pursued based on the interests of the principal investigator (PI), their curiosities, and the questions they wanted to address. We encouraged all of our group leaders to have at least one project that's translational in nature. That's why we called it TSL+, as it represents an added layer of research and science on top of the blue-sky research.

The fascinating thing about this approach is that it created a sort of pipeline. I'll provide examples of how the basic research can lead to applications that develop and grow into significant collaborations with industry. But what's also truly remarkable about this is the "gained in translation" aspect of it. I'll highlight one or two cases where the translational, let's call it problem-driven research, initially started because of a problem or collaboration with industry, resulted in findings that led back to new hypotheses and new basic research ideas. For me, this has been extremely exciting in the last 10 years or so.

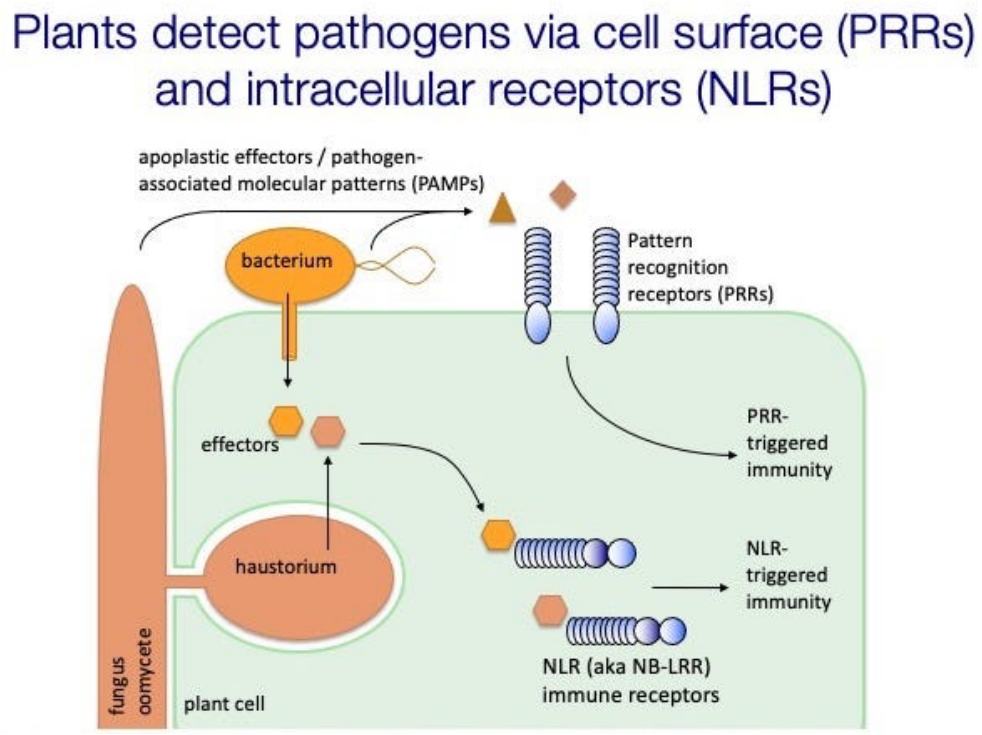


A deep dive into the biology of NLR immune receptors

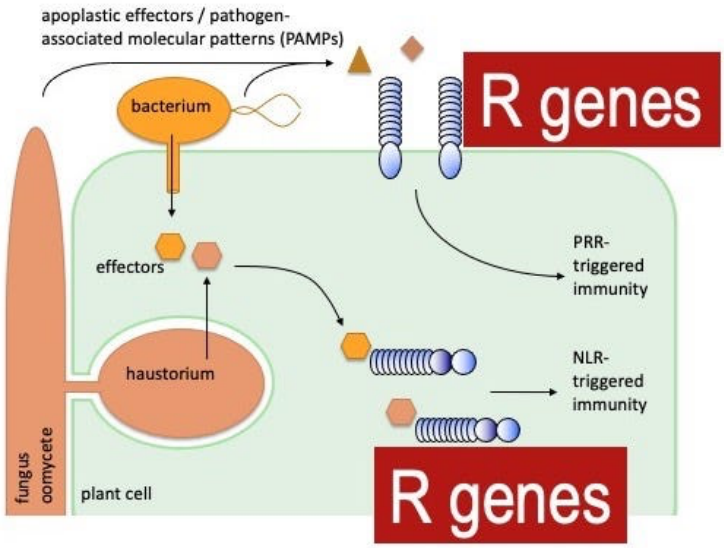
Now, returning to this robust conceptual framework we have in plant immunity, which is the collective work of dozens of groups, we know that plants have two types of immune receptors. These are biochemically quite different.

The first type are the cell surface receptors, known as PRRs or Pattern Recognition Receptors. They are situated on the cell's surface and they detect apoplastic effectors or extracellular molecules derived from the pathogens.

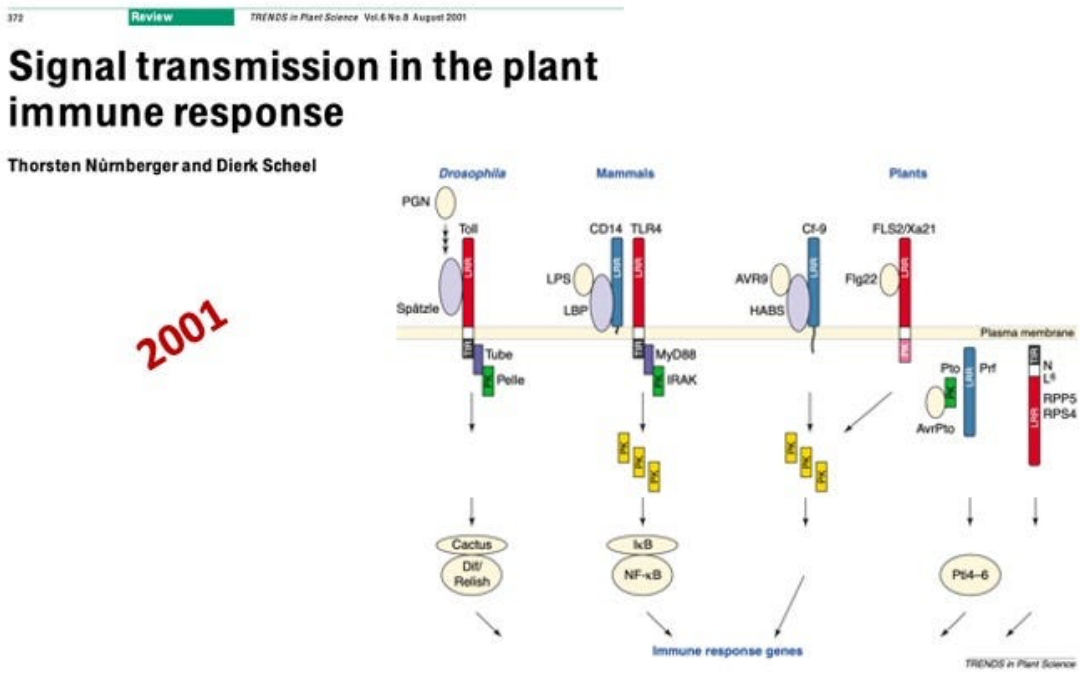
The second type are the intracellular receptors, referred to as NLRs or Nucleotide-binding Leucine-rich Repeats proteins. NLRs are a fascinating family of immune receptors found in humans as well. For example, if you have Crohn's disease, that's an autoimmune mutation in an NLR receptor. NLRs were also recently validated in bacteria as having antiphage activities. In plants, they function as intracellular sensors that can detect pathogen effectors. They tend to be extraordinarily diverse in plant germplasm.



Those of you who approach the field more from the perspective of genetics or plant breeding would be aware that the so-called R genes, which have been known for over a century through classical breeding and genetics, tend to code for these immune receptors. Most often, these immune receptors, whether they are cell surface or intracellular, are the products of these R genes.



About a month ago, I was in Halle for a memorial symposium in memory of Dierk Scheel, who sadly passed away around a year ago. Dierk and his long-term collaborator, Thorsten Nuernberger, wrote a review article about 20 years ago. This review depicted the working models of the time. In the early 2000s, roughly a decade after the cloning of the first R genes, people began to understand that these genes resembled receptors. The different classes of receptors, such as cell surface and intracellular ones, were being identified. At that time, the working model was quite straightforward. It was understood that ligands were detected by these receptors, which activated a signaling pathway leading to a transcriptional response, and subsequently, an immune response. That was the understanding 20 years ago.



We now understand that the earlier model of plant immunity was somewhat naive. Things are more complicated than initially believed. However, that model served as a good foundation for many robust research projects. As it stands, the model has become more complex. I will highlight a couple of the key findings that took us beyond that initial model presented by Thorsten and Dierk.

One significant finding is that the immune receptors themselves form an immune network. While every signaling pathway is indeed a network, in this case, the biochemically related receptors function collectively as a network. This has profound implications, as I will illustrate later.

We now understand that there are different layers of receptors. Some are directly involved in sensing the pathogens, while others, termed co-receptors or helpers, are involved in executing the immune response. These co-receptors interact with the sensors, leading to their activation.

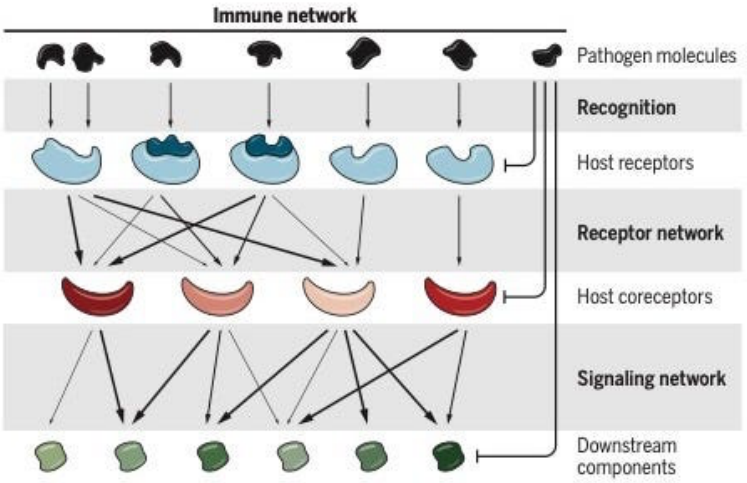
PLANT PATHOLOGY

Receptor networks underpin plant immunity

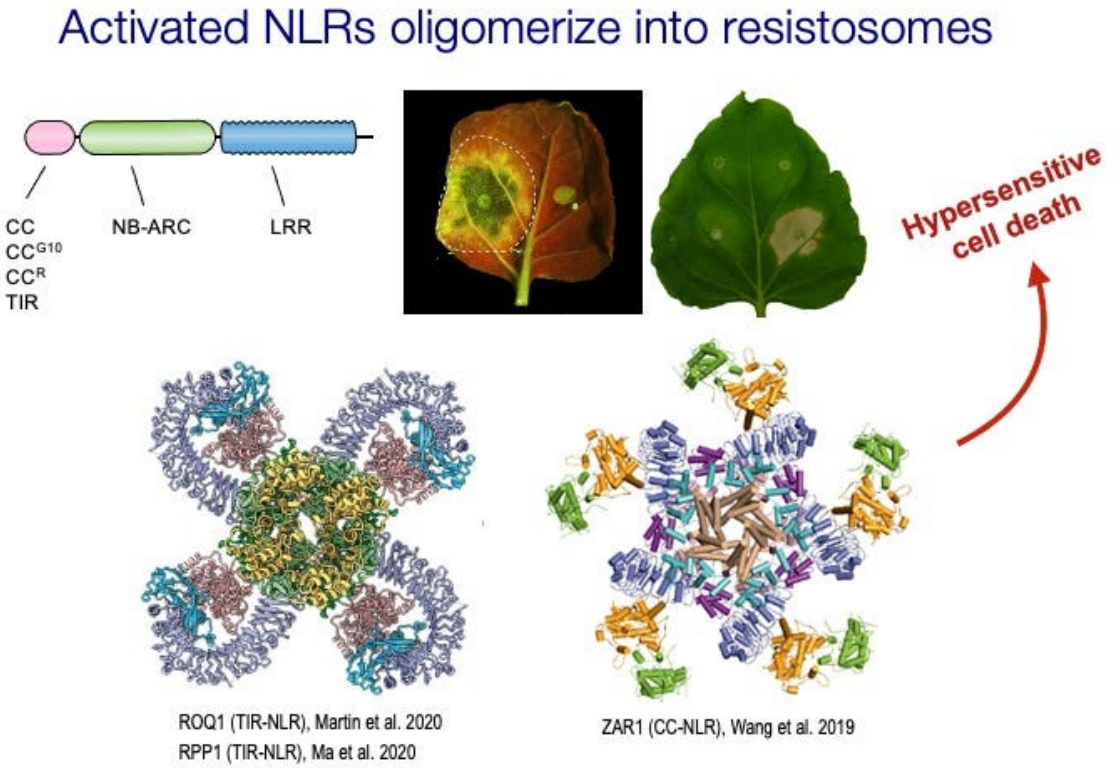
Plant-pathogen coevolution led to complex immune receptor networks

By **Chih-Hang Wu, Lida Derevnina, Sophien Kamoun**

2018

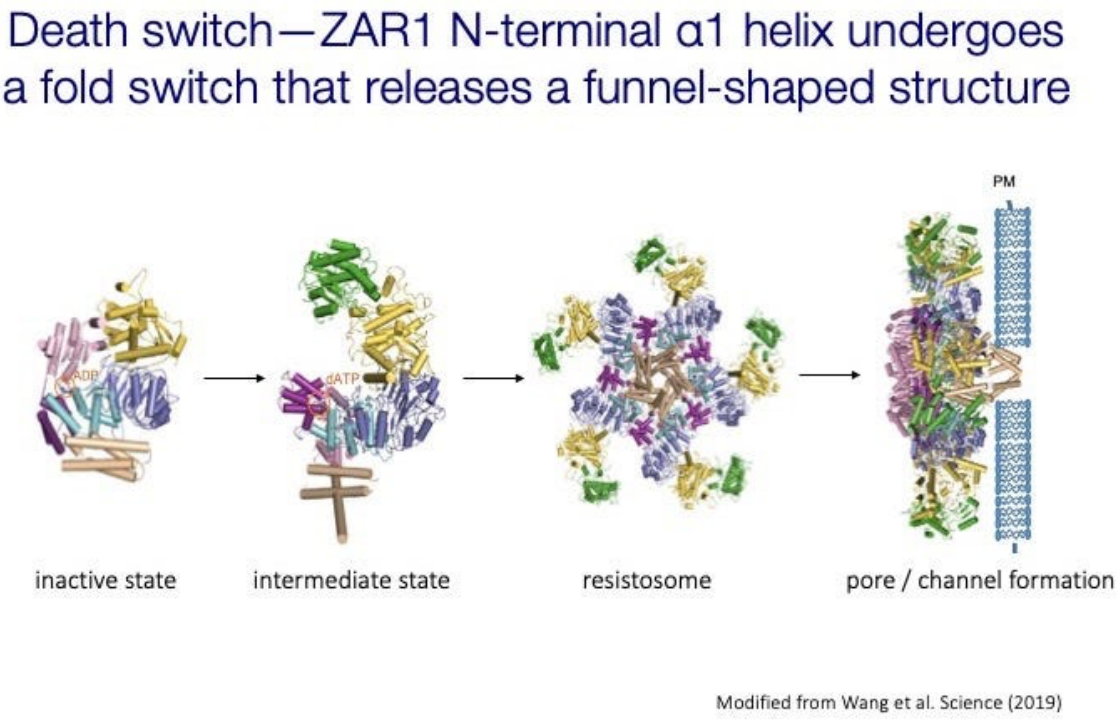


That's where we stand 20 years later. Additionally, a remarkable finding came out of the work from our colleagues in Beijing, specifically from Jianmin Zhou and Jijie Chai. In 2019, they unraveled how these NLRs, the immune receptors, get activated. They studied an Arabidopsis receptor called ZAR1, which responds to bacterial pathogens. They showed that when ZAR1 is activated, it oligomerizes into structures that we call resistosomes.



This resistosome is a pentameric structure. Five ZAR1 proteins come together to form this wheel with funnel-like structure, and the formation of the resistosome is absolutely essential for the activation of the immune response. This is the current model we operate with.

When ZAR1 is activated, a dramatic conformational shift occurs. The alpha helix, the N-terminal alpha helix or alpha1, is normally buried inside the structure of the inactive ZAR1. However, upon activation, it flips out and forms a funnel-like shape that associates with the plasma membrane. This resistosome actually functions as a channel.

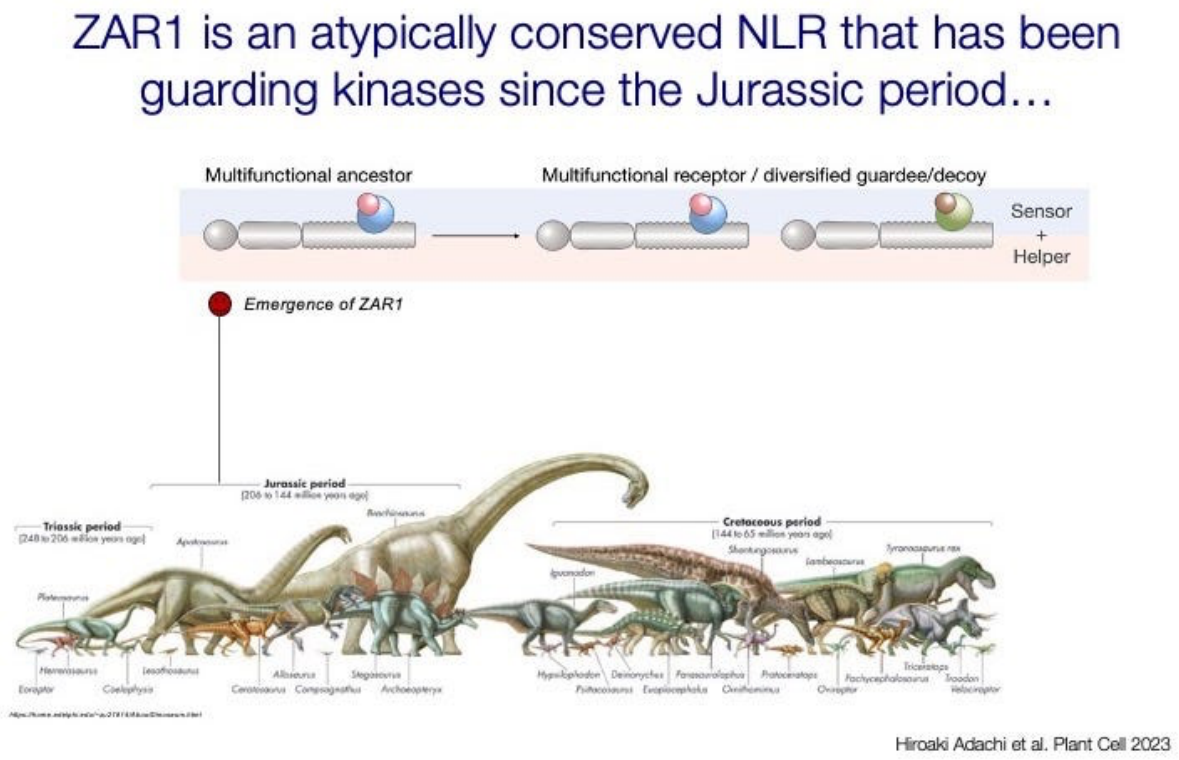


It's now known to be at least an anion channel. We know that calcium travels through this channel, but it could also be disrupting the membrane and forming pores of different types. This discovery would have been impossible without the use of cryo-EM technology.

This discovery really shook the world of plant immunity because it deviates significantly from the older model of a signaling cascade with a MAP kinase pathway and so on. Of course, once this resistosome is formed, many things can occur indirectly, but this provides a very direct route to the immune response that doesn't necessitate a signaling cascade.

ZAR1, though, is quite atypical. We often refer to ZAR1 in our lab as a 'Jurassic NLR' because ZAR1 has orthologs across most angiosperms. This is unusual for an NLR as most NLRs are rapidly evolving and tend to diversify even within a species.

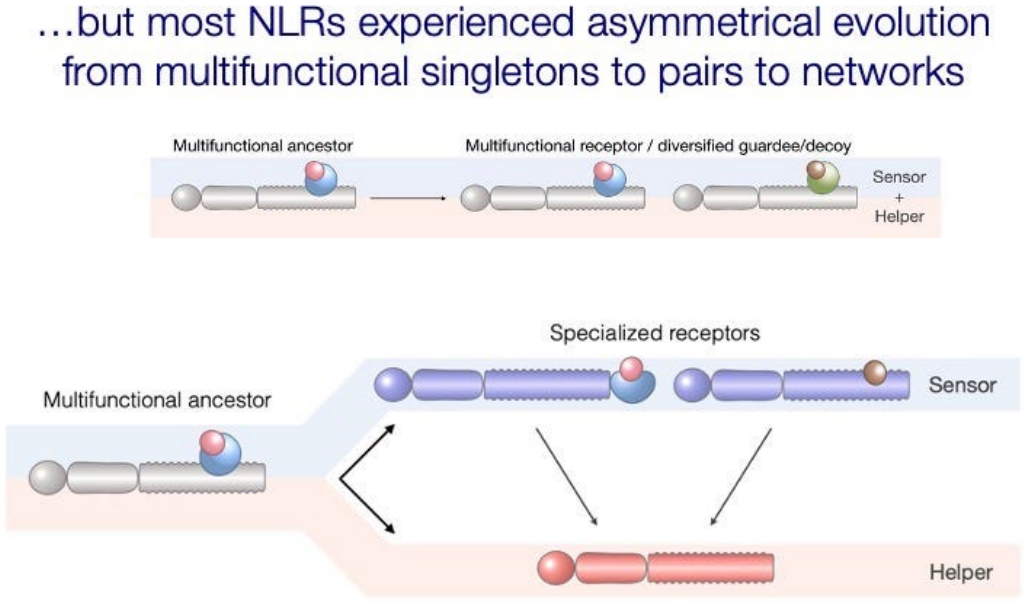
ZAR1 has remained conserved for over 150 million years. It was around when the dinosaurs roamed the Earth. What's fascinating about ZAR1 is the co-evolution that's taken place with the pathogens through the kinases that pair with ZAR1. These are decoy pseudo-kinases that also date back 150 million years and have co-evolved with pathogen effectors.



In this case, the sensing activity is carried out by these pseudo-kinases kinases of the so-called RLCK family, a different protein molecule from the NLR. The NLR is executing the response and detecting the pathogen through the “decoy” kinase.

Evolution of receptor networks

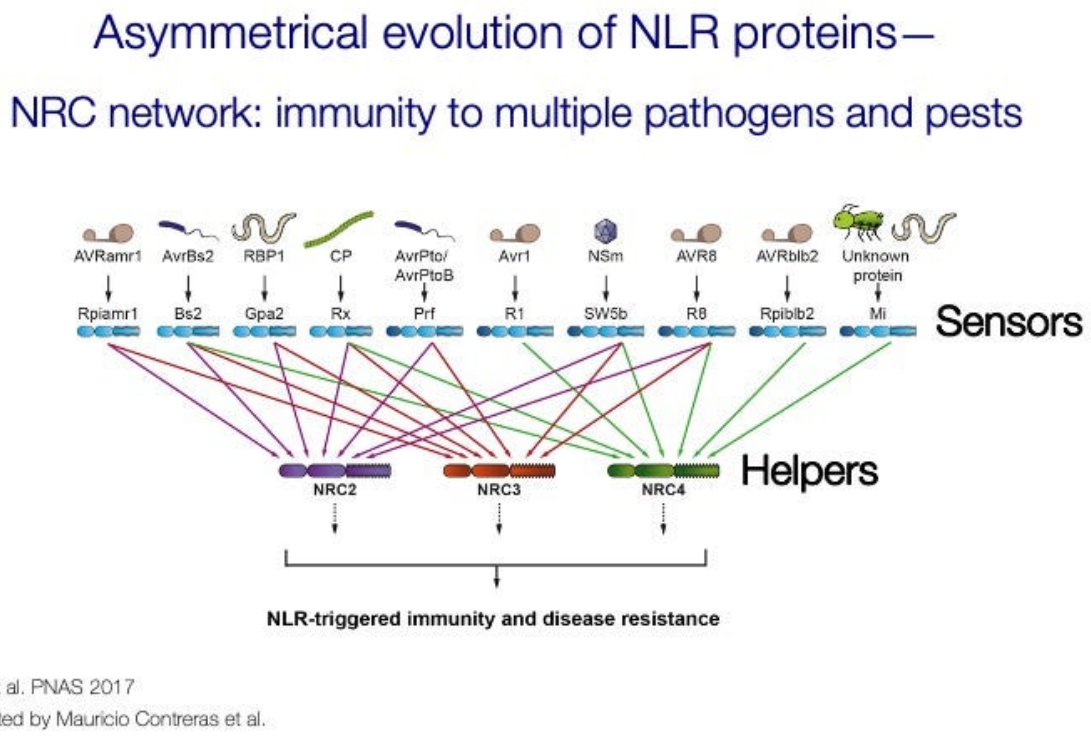
ZAR1 is atypical. The majority of plant NLRs have diversified from this ZAR1-like, multifunctional ancestor into specialized receptors.



This diversification and specialization of plant NLRs have given rise to sensors and helpers: the sensors detect pathogens, and the helpers downstream execute the immune response. This division of labor has occurred repeatedly in the evolution of plant NLRs. In some instances, a single gene has duplicated and then sub-functionalized into sensors and helpers. In other cases, two unrelated NLRs have come together and begun functioning as a pair or even as a network.

This concept came to light through a translation accident, underscoring the interconnectedness of research. This was the work of Chih-hang Wu, a researcher who was in our lab a decade ago. He is currently at the Academia Sinica, running his own lab at the Institute of Plant and Microbial Biology.

The investigation into Rpi-blb2, a plant resistance gene, led us to the intriguing discovery of NLR networks. We were studying this gene in collaboration with BASF Plant Science, hoping to identify its matching effector. BASF Plant Science at the time was developing a potato variety, Fortuna, that carries Rpi-blb2. Chih-hang, then a PhD student in the lab, focused on what was downstream of Rpi-blb2. Remarkably, he found that there was only one gene downstream, NRC4, which was an NLR phylogenetically related to Rpi-blb2.

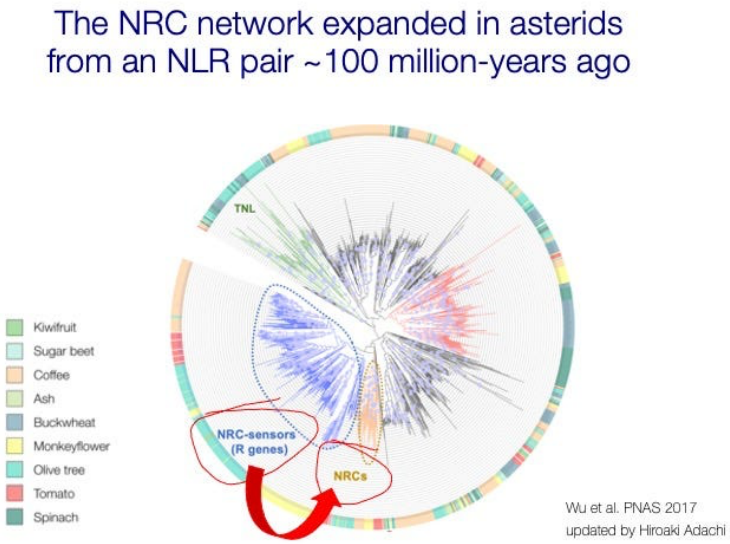


This was indeed a fortunate find. For comparison, similar investigations into say the Rx gene, which provides resistance to the *Potato virus X*, revealed redundancy downstream with NRC2, NRC3, and NRC4 functioning as Rx-helpers. This redundancy makes the task of pinpointing the exact gene involved a challenging one if we rely on traditional genetic approaches. This is why it took about 20 years to discover the downstream NLR helpers.

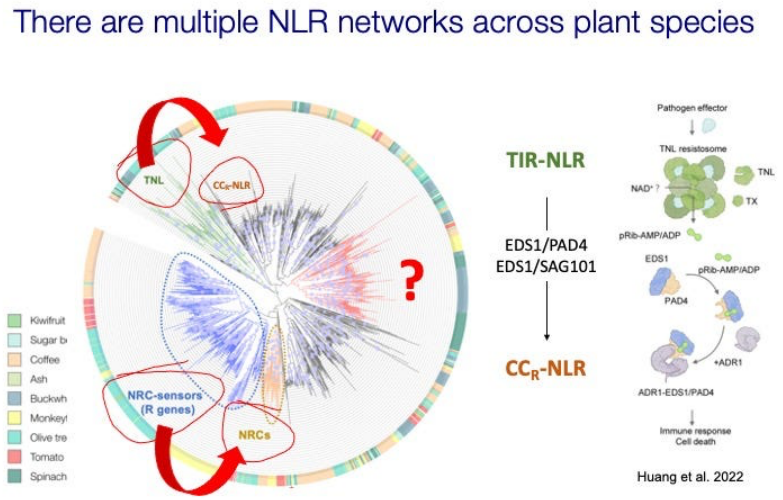
Chih-hang's work with Rpi-blb2 and NRC4 exemplifies how research intended for practical application can serendipitously uncover fundamental insights. He found a phylogenetic

structure within the network. Specifically, all the ‘R’ genes or resistance genes (the sensors) were in one clade, phylogenetically close to the NRCs (helper NLRs). This implies that each of the sensors from this clade requires a helper gene from the NRC clade to function effectively.

This research suggests that the NRC network we are seeing today has evolved from a single NLR gene around a hundred million years ago, early in the evolution of asterid plants. This solitary gene duplicated into separate sensor and helper genes, which then greatly expanded over the course of evolution. In some asterid species, as many as 80% of the NLR genes are part of this network, indicating an extensive degree of diversification within this gene family.



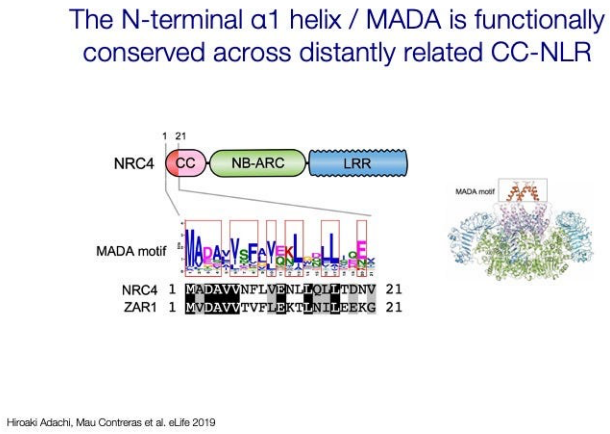
This incredible phylogenetic structure underpins the diversification of NLR genes and their networks. Additionally, there are other networks in plants. For instance, my colleagues Jijie Chai and Jane Parker have discovered the TNL-to-CCR-NLR (or RNL) network, another phylogenetically structured network. In this network, every TNL (Toll/interleukin-1 receptor-Nucleotide binding site-Leucine-rich repeat) seems to require a downstream CC-NLR (Coiled-Coil Nucleotide-binding site Leucine-rich repeat) to execute the cell death response. These downstream helpers all belong to a single phylogenetic clade in the plant NLR tree.



We believe there are likely more of these networks to be discovered, particularly in monocots, a major group of flowering plants that includes grasses and lilies, where there are already several indications of such networks.

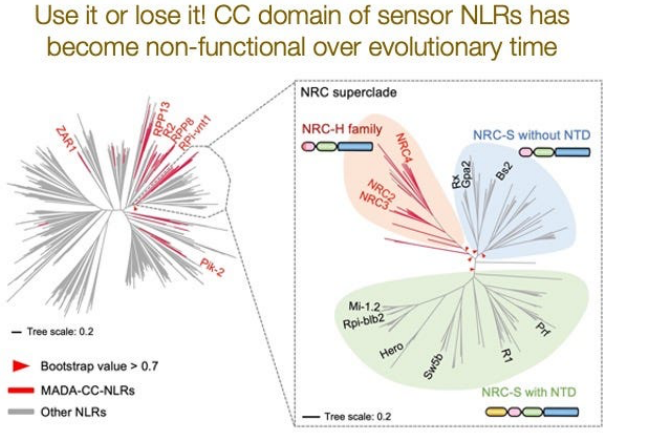
NLR diversification: Use it or lose it

In the evolutionary process of diversifying into networks and sub-functionalizing into sensors and helpers, NLR genes underwent significant changes. The insight from the study of the ZAR1 protein was particularly enlightening in this context. As mentioned, the very N-terminal alpha helix portion of this protein is critically important for its function, particularly as a channel for calcium ions.



What's remarkable is that this alpha helix is conserved across NLRs from many diverse species, even as far back as liverworts, which are non-flowering plants. It is identifiable by the MADA sequence motif. However, the MADA-type alpha helix is conserved in only about 20% of NLRs. The majority of them, especially the coiled-coil NLRs (CC-NLRs), do not have it.

It appears to be a classic case of evolutionary "use it or lose it." That is, because the majority of CC-NLRs no longer needed this alpha1 helix for their functions, it was lost over time. This highlights the impact of evolution in shaping proteins and biological systems according to their functional requirements.



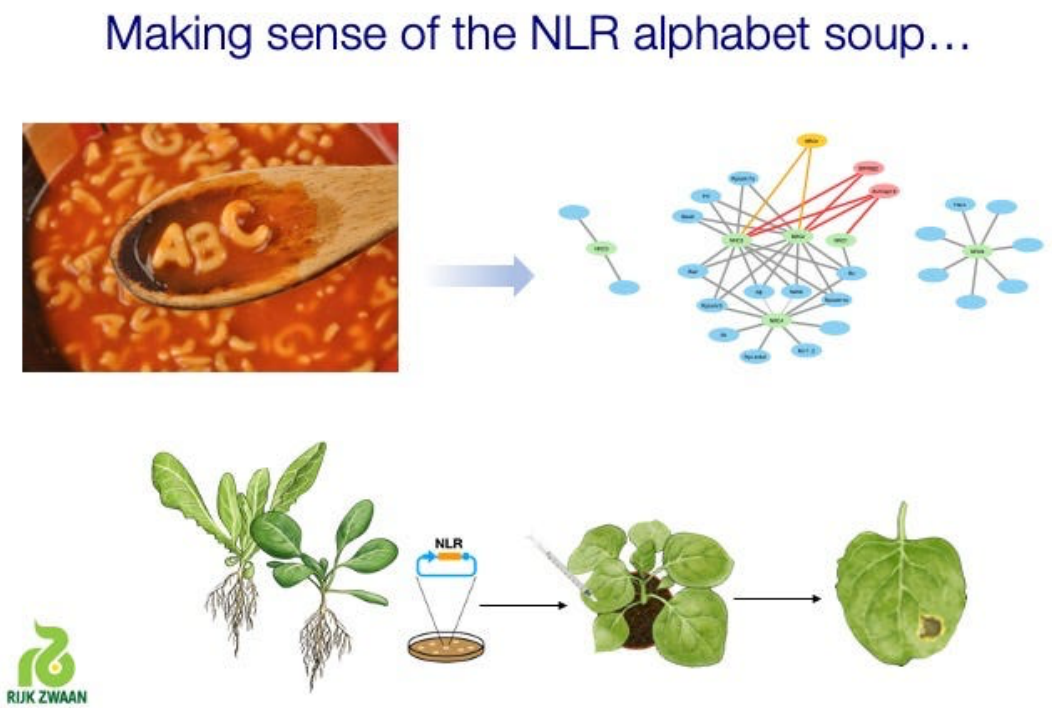
Over time, most of the sensor NLRs have lost the MADA sequence, indicative of the alpha-helix structure N-terminal structure, which is critical for immune functions, such as forming a channel for calcium ions. The MADA sequence is still conserved in helper NLRs, demonstrating their role in the direct execution of immune responses.

Interestingly, a subset of sensor NLRs, including Rpi-blb2 mentioned earlier, have acquired an additional N-terminal domain integrated before the CC domain. This new domain is involved in recognizing and baiting the pathogen. In other words, these sensors have fully lost their ability to function like a resistance protein that executes cell death. Instead, they rely on the helper NLRs to carry out these functions.

This is a great example of how evolutionary information can enhance our understanding of the biochemical activity and mechanistic functioning of proteins.

Making sense of the NLR alphabet soup

This work may seem fundamental in nature, but it has important practical applications, because understanding the NLR network architecture of a particular plant species has implications, for example, in plant breeding.



This is exactly why we collaborate with Rijk Zwaan, a vegetable seed company, to work with their crops of interest. They have genomes for lots of crop plants, and we're trying to make sense of that alphabet soup of NLRs.

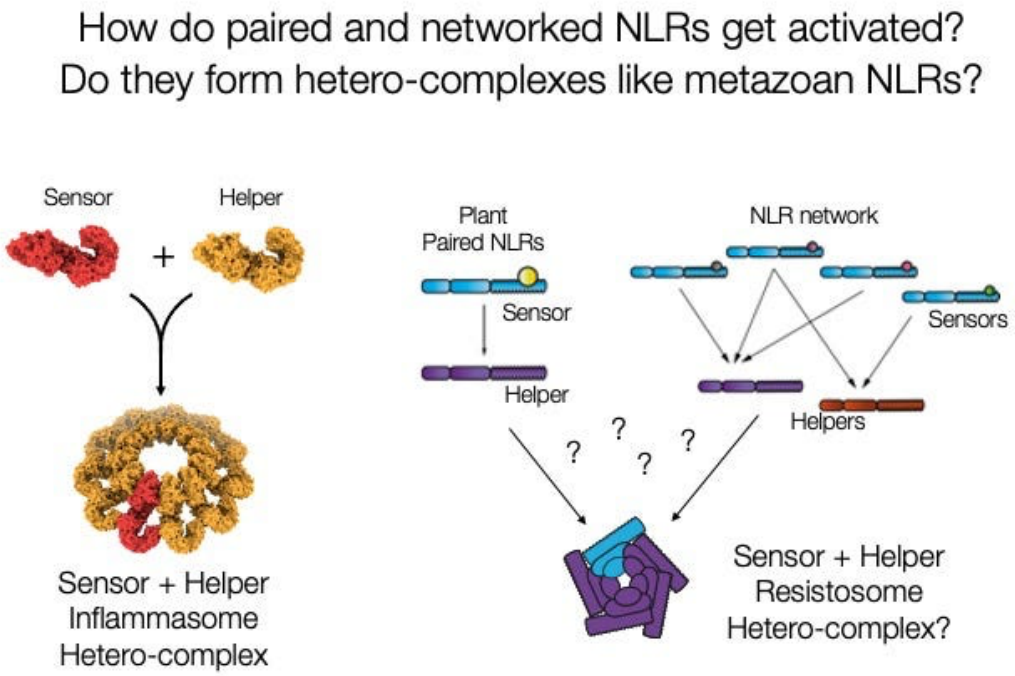
You sequence a typical plant genome, you end up with several hundred NLRs. You need to make sense of that. So, we're using information from phylogenomics, from gene expression,

from location in the genome, and so on, to start building the networks. And we're also experimentally validating them using model systems.

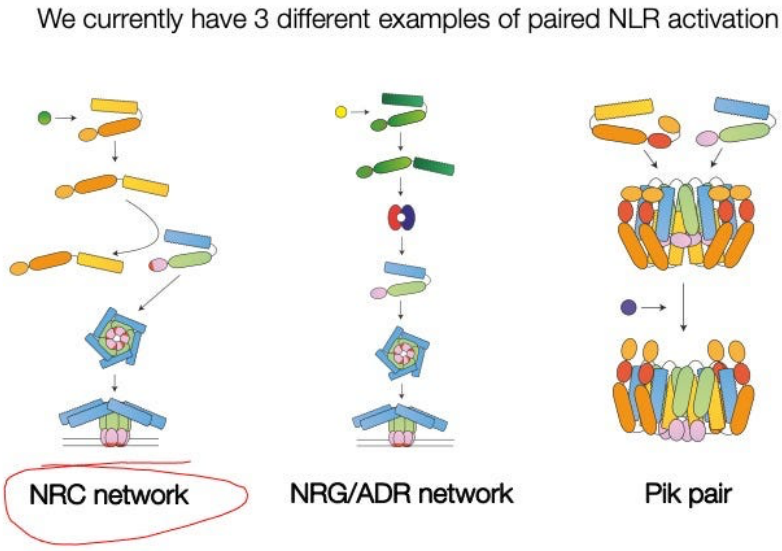
And then these nodes in the network become really critical in terms of breeding, because if a node is missing, then good luck breeding one of these R genes from one germplasm or one wild species to an elite genotype. This is an example where fundamental understanding of NLR biology has real-world practical applications.

How do NLR pairs function?

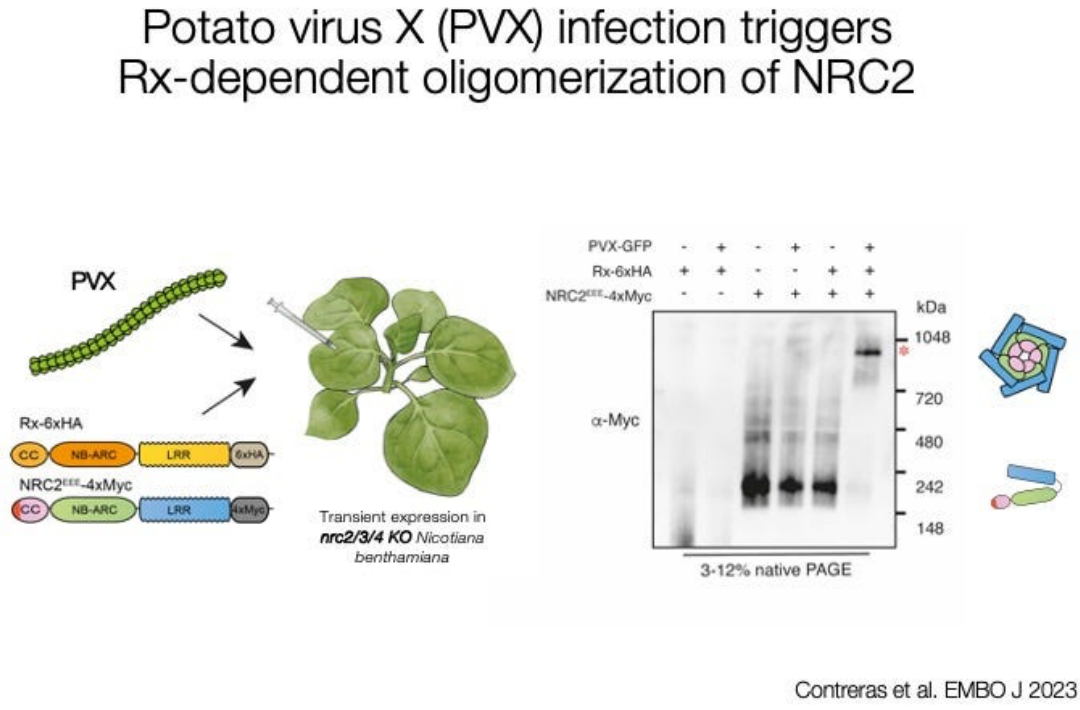
The currently mechanistic understanding of NLR functioning is tied mainly to ZAR1, which is a functional singleton that doesn't require other NLRs to function. The question then arose: how do paired and networked NLRs function? In mammalian systems, paired NLRs can form these hetero-oligomers called inflammasomes, homologous structures to resistosomes. But in plants, how does that work?



I'm going to give you the answer right away. In plants, they work in different ways. For example, here is our NRC network, and I'm going to tell you a little bit about how paired NLRs work within the NRC network. In the NRC network, it's the helper that forms the resistosome, not the sensor.



This is the experiment illustrating the mechanistic model using NRC2, one of our helpers, which we activate with Rx, the gene that detects *Potato virus X*. We assay the system with non-denaturing native gels, which can visualize protein complexes without disrupting them. When the activation has occurred, when we have the virus and the R genes and the whole system in there, we see this pentamer sized oligomer of NRC2, which is similar to what we expect based on the ZAR1 model. On the other hand, nothing happens to Rx. Rx remains unchanged and does not enter into hetero-oligomerization with NRC2.



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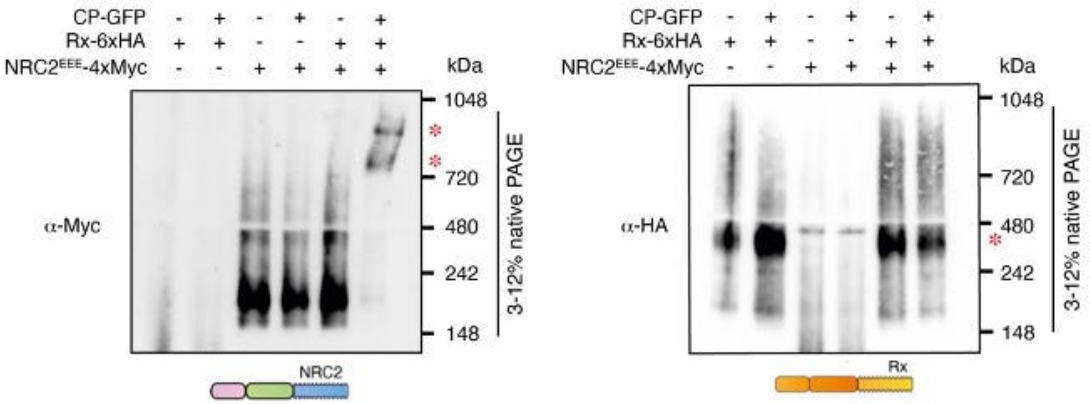
Rx remains unaltered following activation



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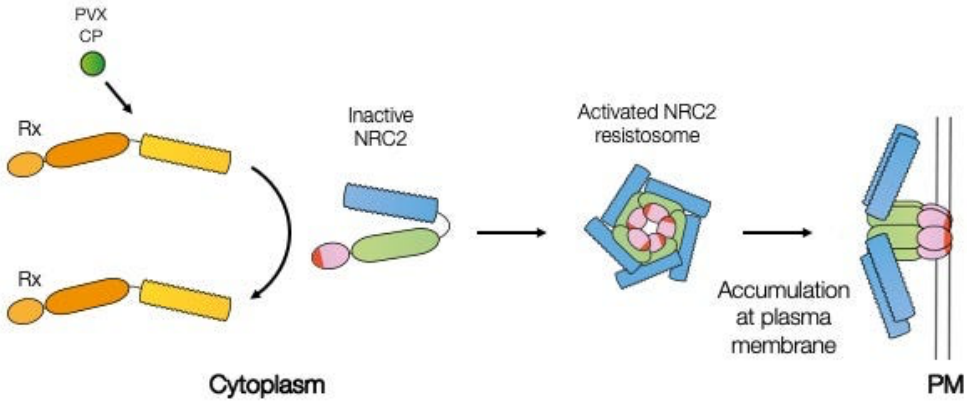
We also have independent cell biology experiments that are consistent with this model. What we think is happening is an activation-and-release mechanism, where Rx is transiently interacting with NRC2, activating it, then NRC2 goes on to activate other NRCs to form the resistosome.

Activation-and-release model for sensor-helper activation in the NRC network



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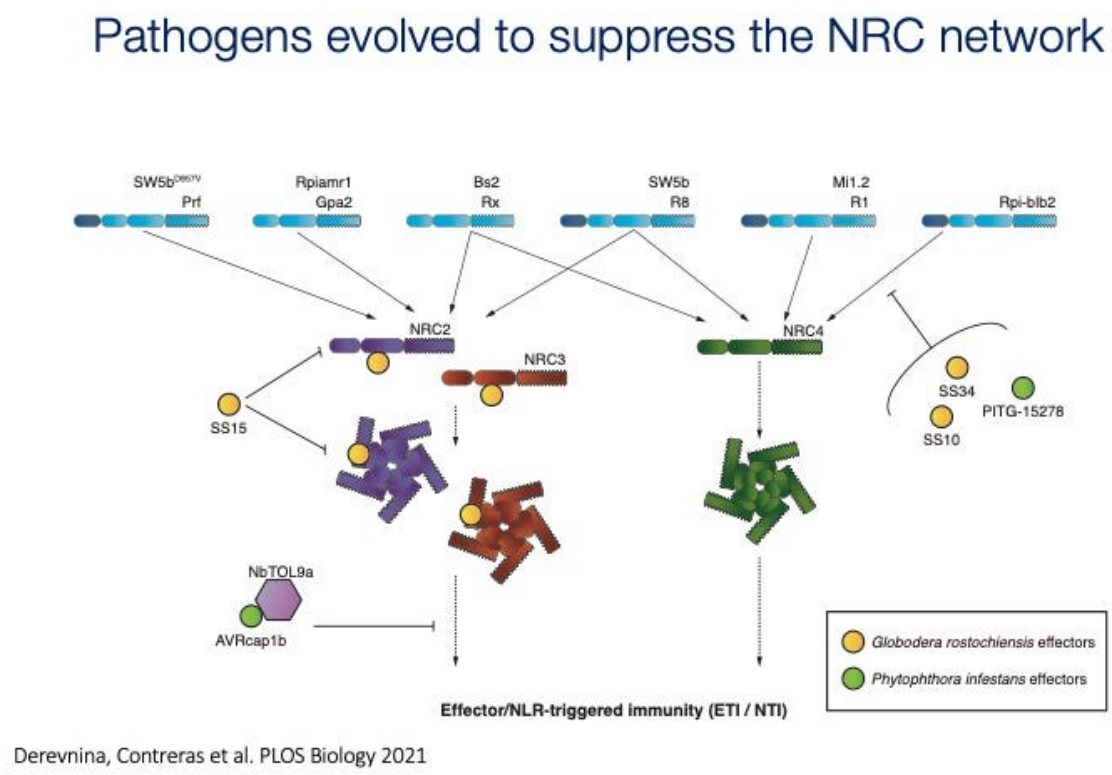
Here too, it looks like the sensor NLR, Rx, has lost the ancestral capacity to form a resistosome, relying instead of its helper NRC2 to oligomerize and execute the immune response.

Pathogen suppression of NLR networks

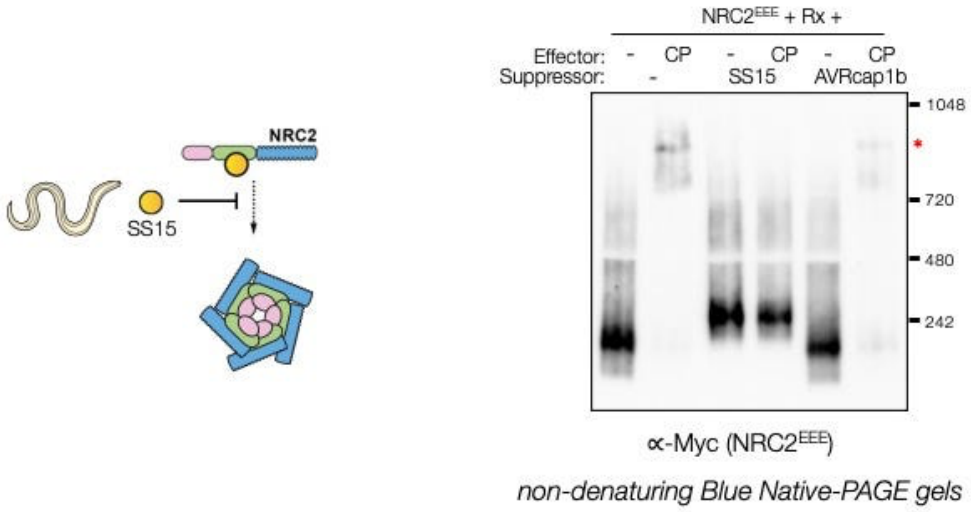
The network model also carries practical implications. Consider this: we have a bowtie-like network with numerous R genes, many sensors, all functioning with a few downstream nodes.



Derevnina, Contreras et al. PLOS Biology 2021

These nodes are optimal targets for pathogens to inhibit and suppress because if the pathogen can incapacitate some of these NRCs, they'd be suppressing a multitude of R genes, essentially dodging a large part of plant immunity. This is the approach we adopted with a postdoc at the time, Lida Derevnina. When Lida joined the lab, she implemented screens in search of pathogen suppressors of the NRC network, and she discovered several suppressors. One that turned out to be particularly interesting is from the potato cyst nematode. This effector directly binds to the NRC protein.

Nematode effector SS15 blocks NRC2 oligomerization



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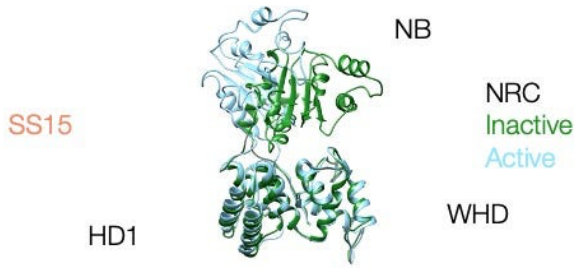
Not only does the effector binds to the NRC, but it also locks it into an inactive state. As you can see here in this complex, normally, NRC2 oligomerizes following activation. However, the nematode effector inhibits NRC2 by forming a complex with it and preventing its oligomerization.

We successfully obtained the structure between the effector and the NRC. When observing the NRC, note how its conformation changes during oligomerization. Please pay attention to this hinge here, which rotates as you can see. This hinge is exactly where the effector binds. We believe it's essentially immobilizing the NRC, preventing it from rotating after activation.

SS15 immobilizes critical hinge loop to prevent NRC activation



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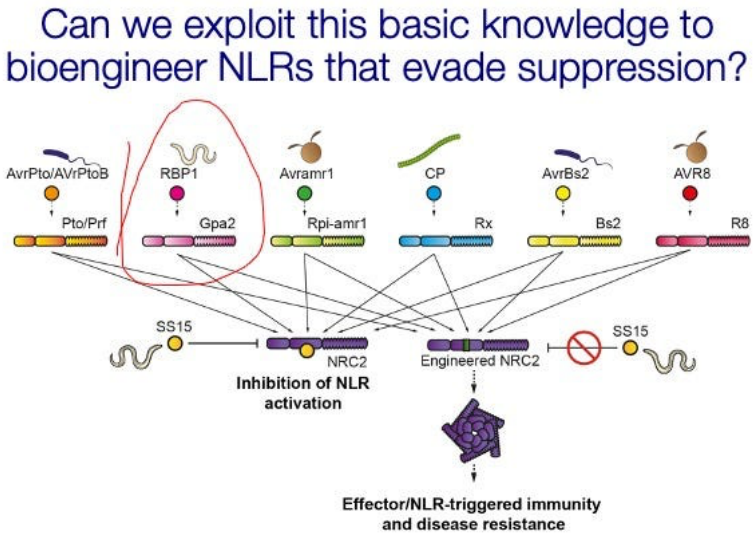
Resurrection of disease resistance genes

This basic knowledge has practical applications, reverting back to our theme of translation, because if you think about it, by understanding exactly how the inhibitor inhibits the NRC, we can introduce mutations into the NRC and engineer, potentially with just a few minor mutations, an NRC that is insensitive to inhibition and binding by that pathogen.



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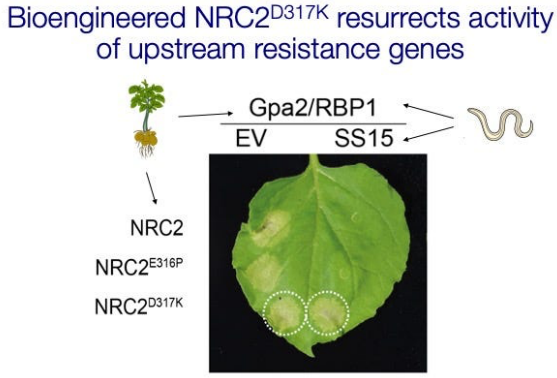
We did exactly that, and I'm going to show you a case where we worked with a nematode resistance gene that is detecting a nematode protein, and that is also part of the NRC network.

Let me take you through this slightly complicated experiment. First, we have a resistance gene from potato, called GPA2, which is dependent on NRC2 to activate the immune response. In a typical immune response scenario, the plant receptor GPA2 detects a protein from the nematode called RBP1 and activates the helper NRC2, which in turn activates immunity. If we introduce the nematode SS15 suppressor, immunity (visualized here as a cell death response or brown tissue) does not occur—the suppressor is doing its job.



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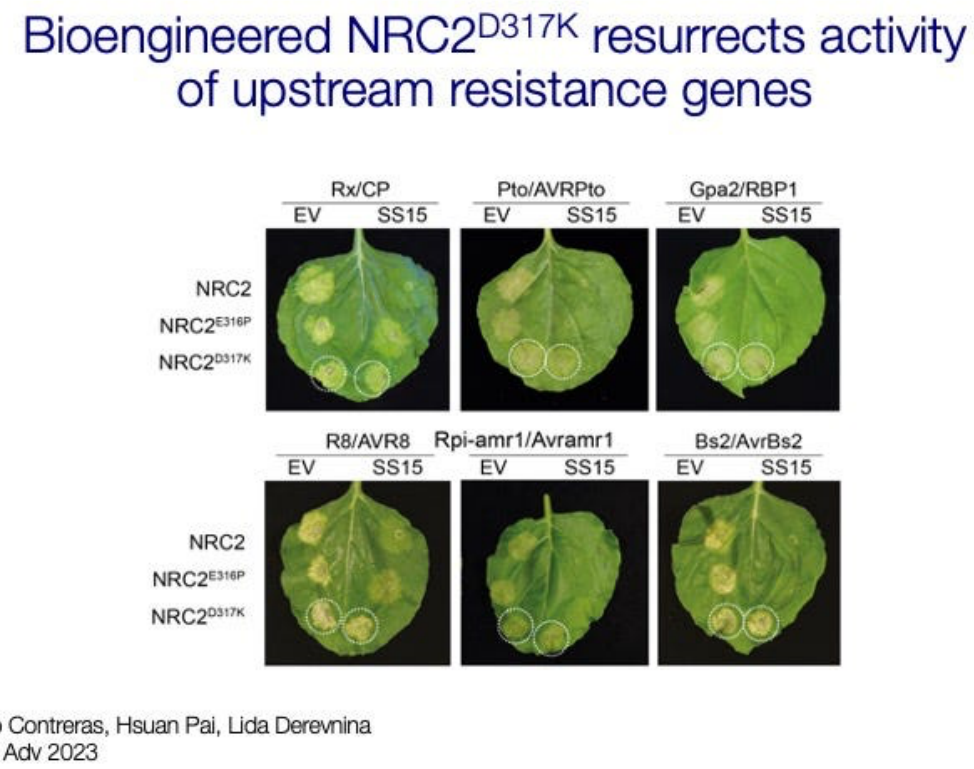
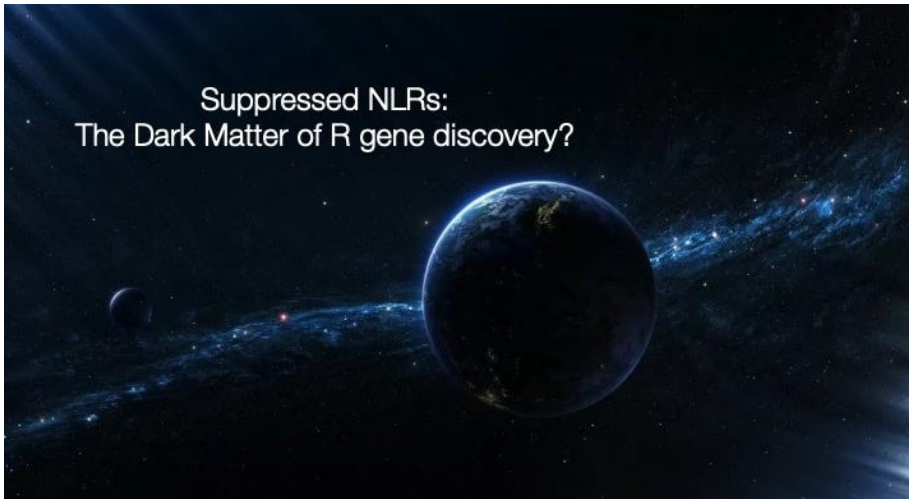
However, if we introduce a single amino acid mutation in the hinge region of NRC2—a mutation designed to evade inhibitor binding—there is no binding of the suppressor, and therefore, no suppression by the nematode effector.

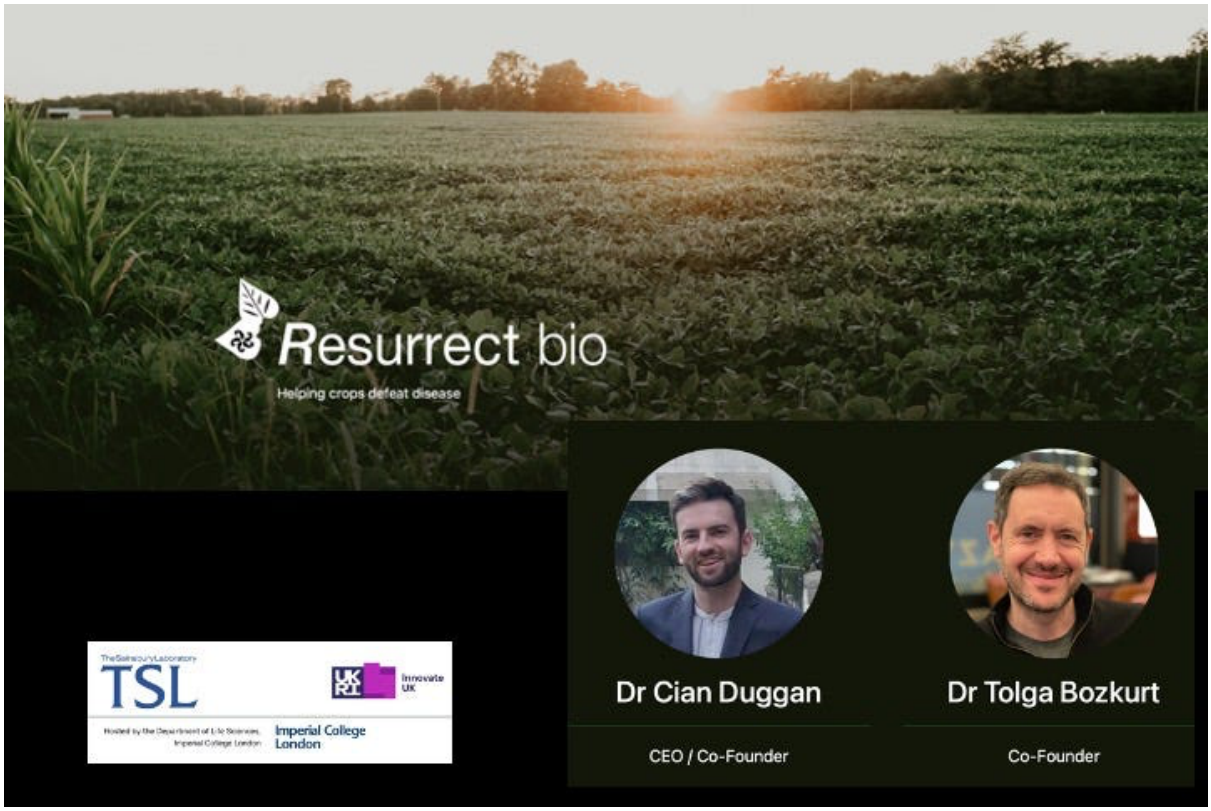
We refer to this as 'resurrection.' We've essentially resurrected resistance genes by bioengineering the helper receptor. And this doesn't just apply to GPA2; it can be implemented with a host of other resistance genes.



What implications does this work have? Could there be a multitude of resistance genes sitting in crop genomes that are typically suppressed by pathogens, which we could potentially resurrect using this method? This intriguing possibility is what drove us to establish a spinout company. I teamed up with my colleagues, Cian Duggan and Tolga Bozkurt, from Imperial College to make this a reality.

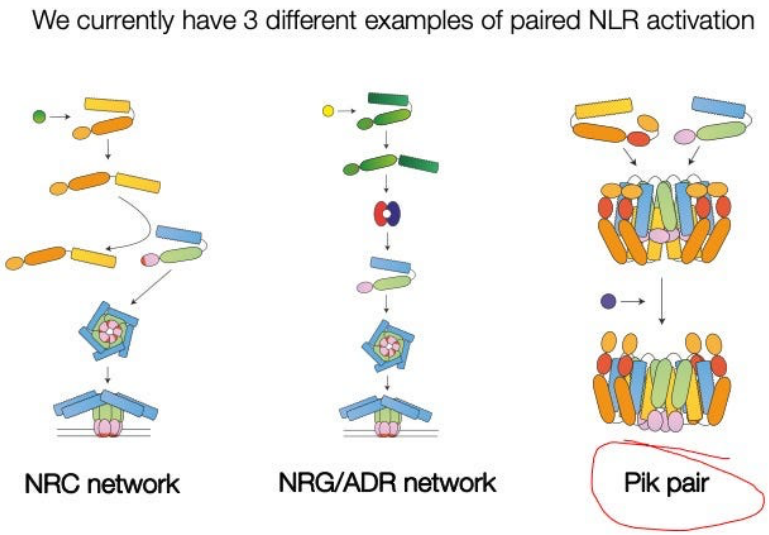


We named our company Resurrect Bio. Cian, who has now fully transitioned to lead the company, has recently secured seed funding of $2 million. His current focus is to test this concept, exploring the extent to which he can revive, or ‘resurrect’, disease resistance in our crops.



Pikobodies: Made-to-order disease resistance

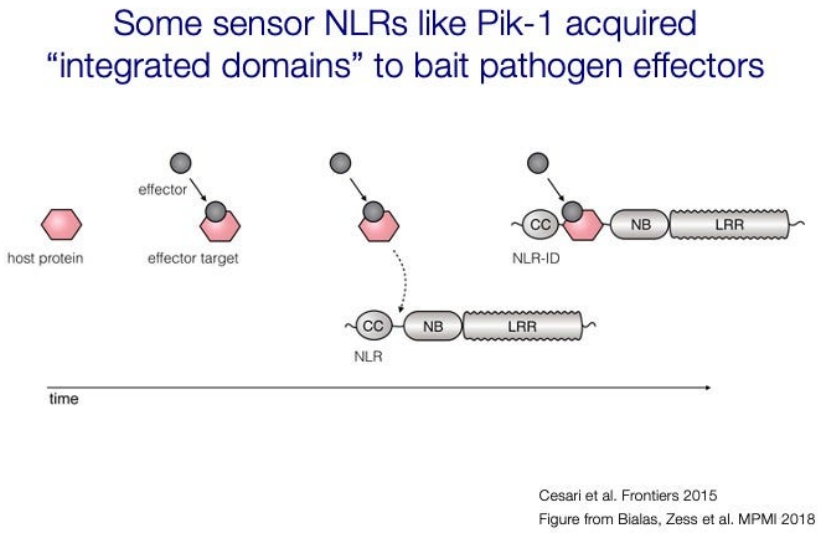
I’d like to touch on the concept of the “Pik pair”. This is another mechanism where paired plant immune receptors function together. In this case, they form a pre-activation complex that probably undergoes conformational changes after activation.



This has proven incredibly useful, as it means the pre-activated complex can be transferred from one plant species to another. Although the Pik genes originate from rice, we know they functions in dicot plants.

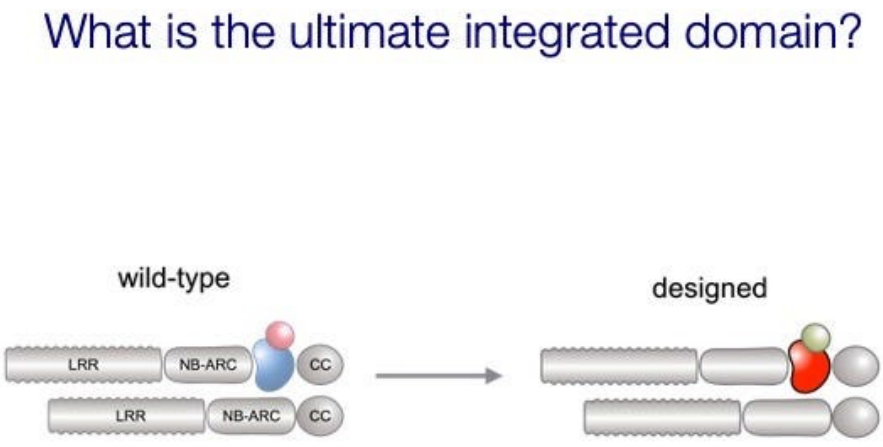
The complex can be transferred from one species to another because it forms a pre-activated biochemical unit. Pik-1, the sensor, is one of these plant immune receptors that has acquired a new domain throughout evolution, baiting the pathogen effector.

This is a concept that was formulated by INRAe scientist Stella Cesari about a decade ago. Stella demonstrated how around 5% of these receptors have an integrated domain that detect the pathogen via what she calls a decoy domain. These domains originate form the targets of the pathogen effectors. Essentially, the plant turned the table on the pathogen, using their effector targets as decoys to detect them and activate immunity.

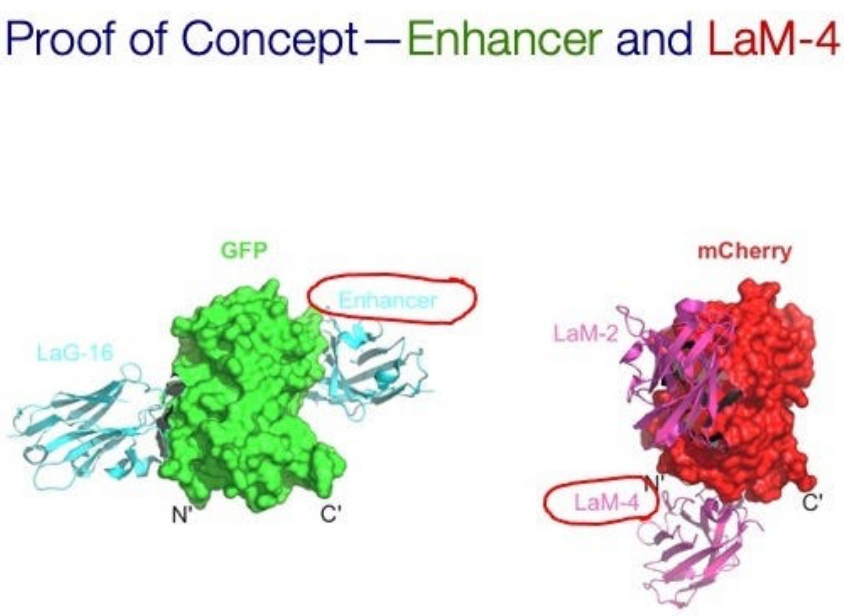


Cesari et al. Frontiers 2015
Figure from Bialas, Zess et al. MPMI 2018

Naturally, as soon as Stella articulated this concept, the question arose—can we recapitulate evolution and design our own resistance genes? And if so, what would be the ultimate integrated domain?

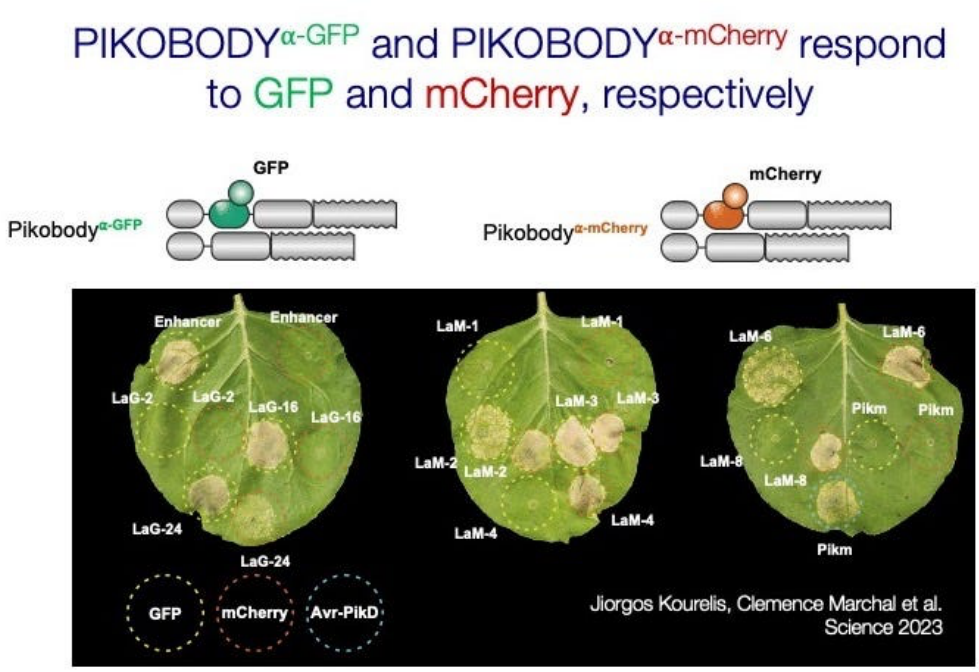


Antibodies! In particular, nanobodies, sequences derived from the antibodies of llamas, camels, and so forth, have a simple architecture—a single domain. We tested the concept of fusing nanobodies with NLRs using anti-GFP and anti-mCherry binders, such as Enhancer and Lam4.

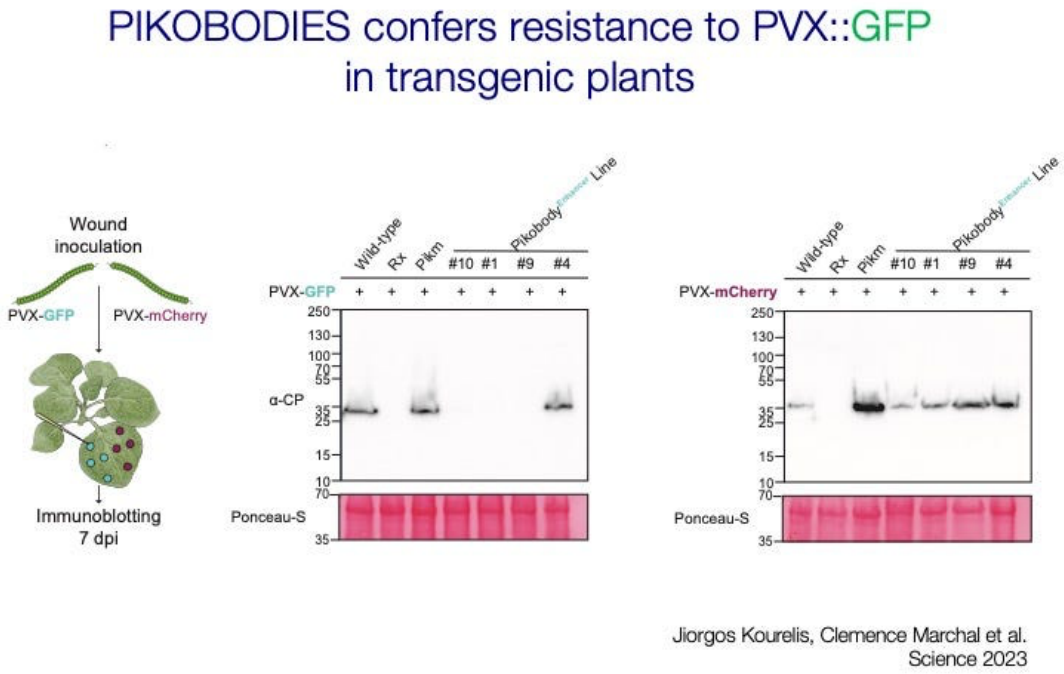


In short, this approach works. We've named this technology Pikobodies. Pikobodies refers to a mix of the Pik plant immune receptors and camelid nanobodies. And here is a playful nod to my home country, Tunisia.

Here is the proof-of-concept experiments. This system operates by activating immunity against GFP, not mCherry, when using the Pikobody that carries the anti-GFP nanobody Enhancer and vice versa. For instance, Lam4 against mCherry triggers an immune response against mCherry, but not GFP.

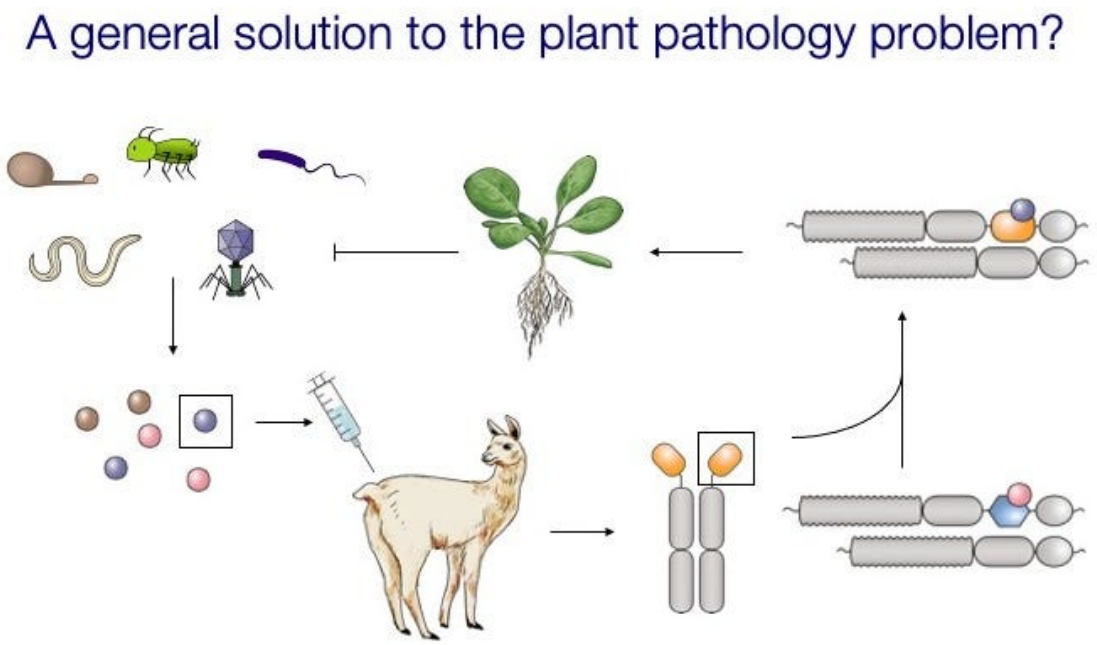


We can also obtain resistance to a virus expressing these proteins. In this experiment, transgenic plants expressing the anti-GFP Pikobody confers resistance to a strain of *Potato virus X* expressing GFP. Interestingly, although the Pik genes originated from rice where they confer fungal resistance, we've discovered that we can use Pikobodies to trigger immunity against a virus in transgenic lines. Some of these lines are entirely immune, similar to the natural resistance gene Rx.



The Pikobodies tech is quite exciting because it essentially provides a pipeline for designing custom-made disease resistance genes against any effector or pathogen we wish to target.

Essentially, we transferred the mammalian vaccination framework to plants. We're currently investigating potential applications for this work.



A bright future for NLR receptor bioengineering

In conclusion, I believe there's a bright future for bioengineering of plant immune receptors, a future that's deeply rooted in fundamental research and discovery. Stay tuned.

Acknowledgements

This post was adapted from the transcript of the Ghent talk with assistance from ChatGPT. I'm grateful to members of my lab and other colleagues for their ideas and for sharing slides and related material.

Appendix

Looking back at TRIC23 and ahead to the future of agriculture.

Gained in translation: Bridging the gap between curiosity and applied research
kamounlab.medium.com