Trends in Plant Science

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Spotlight

Dancing with the Stars: An Asterid NLR Family

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Wu and co-workers show how a network of sensor and helper NOD-like receptor proteins (NLRs) act together to confer robust resistance to diverse plant pathogens.

Plants engage with a plethora of potential pathogens but only some of these microbial overtures lead to disease. This is due to a highly successful system of innate immune receptors that quickly identify the invader and halt its progress. Wu *et al.* [1] now describe new insights into the molecular choreography of plant immune receptors.

Our understanding of these dances began with a simple two-step. There are two partners involved: a *Resistance* (*R*) gene in the host and an *Avirulence* (*Avr*) gene in the pathogen. They dance a dance according to the gene-for-gene model and resistance is manifest only if both partners are present [2]. The simplest interpretation of the gene-for-gene model is that the *R* gene encodes a receptor for the product of the *Avr* gene [3]. In fact, most *R* genes encode NOD-like receptors (NLRs) that pair a central nucleotide binding domain with C-terminal leucine rich repeats (NB-LRR proteins) [4]. On the other side, most *Avr* genes encode effectors that are secreted by pathogens to maintain virulence by strategic manipulation of host targets. As LRRs are receptor moieties in other proteins, early models posited them as receptor domains for effectors in a direct interaction, and this simple model holds true for some resistances [5].

Along the way, it transpired that more sophisticated models groove to a different beat. For instance, many NLRs recognise changes induced in another host target protein that is modified enzymatically by pathogen effector (Avr) proteins [6]. Examples are also known in which decoy proteins mimic such host target proteins and facilitate recognition by NLRs [7]. Effector decoys can also be provided in cis as a fusion with the NB-LRR moieties [8]. Some NLRs dance solo, but others need two to tango. In this molecular pas-de-deux, one NLR partner is the sensor that interacts with an effector, and the other is a helper that stimulates downstream signal transduction events. These pairs interact physically, and strikingly, are typically co-located genomically in a tail-to-tail arrangement (Figure 1) [9].

Sensor-helper relationships also occur between non-linked NLR genes. A widespread class of NLRs called CC_R proteins typified by the *Nicotiana benthamiana* N-required gene 1 (NRG1) and Arabidopsis activated disease resistance gene 1 (ADR1) proteins are needed for a number of sensor NLRs that recognise diverse pathogens [10]. However in this case no contact between sensor and helper has been reported. An analogous situation exists in the Solanaceae, the nightshade family, which includes tomato, eggplant and tobacco. Here a family of NLRs called NRCs (NLR required for cell death), are essential for the function of a range of sensor NLRs [11,12]. Wu and colleagues now flesh out the details of a network of sensors and helpers in Solanaceae that may enhance the robustness of immunity signalling pathways [1].

The economic and cultural importance of the Solanaceae has led to the cloning of over 20 *R* specificities from different species, each conferred by a sensor NLR. The *NRC1* gene was originally defined in tomato as required for the function of several *R* specificities, interestingly including those conferred by both intracellular and extracellular immune receptors [11]. In subsequent work, the Kamoun laboratory described *NRC2* and *3* in the Solanaceous experimental model *Nicotiana benthamiana* which are redundantly required for function of the Pto/Prf NLR complex [12]. The present work expands analysis of the NRC family in *N. benthamiana*, which contains 9 members but no direct ortholog of tomato *NRC1*. Wu et al not only show that *NRC4* is required for *Rpi-blb2* immunity against the oomycete *Phythopthora infestans*, but that a panel of eight NLR genes were dependent on NRCs. The sensor *R* genes *Rpi-blb2*, *Mi1-1.2*, and *R1* require the helper *NRC4* specifically for function, whereas *Pto/Prf* has a redundant requirement for *NRC2* or *NRC3*, and *Sw5b*, *R8*, *Rx* and *Bs2* require one of *NRC2*, *3*, or *4*. These observations point to the presence of complex signalling relationships in which sensor proteins encoded by specific NLRs have variable requirements for members of a group of helper proteins comprising NRCs 2, 3 and 4. Intriguingly, phylogenetic analysis revealed that these NLRs are all members of a superclade that includes the NRC gene subclade. Strikingly, five *R* genes outside of this superclade do not require *NRCs* for function. Thus, NRCs appeared to have seeded both the sensor (NRC-S) and helper (NRC-H) sides of an expanded family of interacting NLR sensors and helpers in an evolutionary dance that spans aeons.

So where did the NRC superclade come from? A higher order phylogenetic analysis found that it is absent in rosids (including strawberry, soybean, and the geneticists' favourite, Arabidopsis), but represented in sugar beet (Caryophylales) and asterids (including the Solanaceae, coffee, and kiwifruit, amongst others). Within the analysed species, there is considerable variation in the number of *NRC* genes, which can be parsed into NRC-S (sensor) and NRC-H (helper) clades based on phylogenetic affinity. It is greatly expanded in most asterids, but present in a very limited form in sugar beet and kiwifruit. This key insight allows a notional

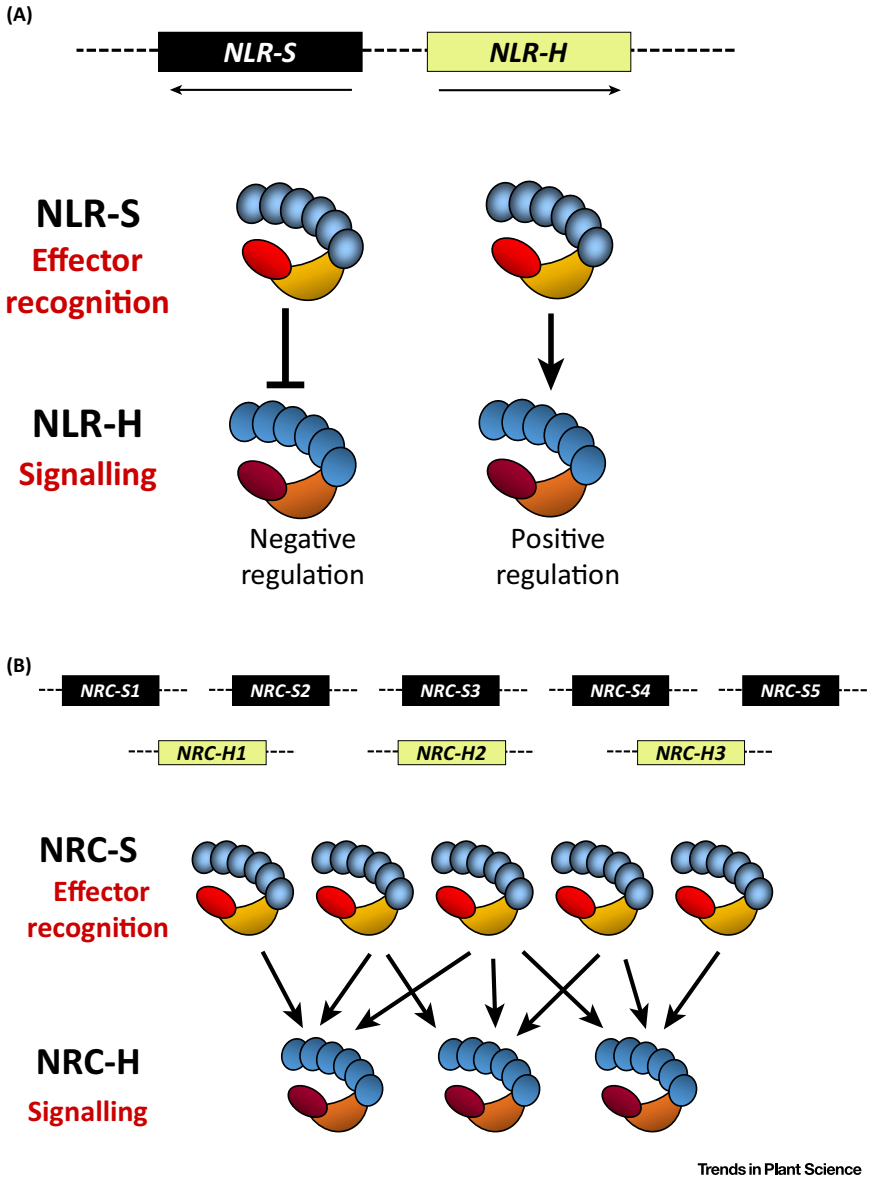

Figure 1. Evolution of NRCs in Solanaceae. A, Upper; putative ancestral NLR gene pair that gave rise to the NRC superclade. Lower; the original negative regulation of NLR-H by NLR-S evolved to a positive relationship (right) allowing expansion of the NRC clade. B, Upper; expansion of the sensor domain NRCs with different recognition specificities operating through a small number of helper NRCs. Lower; schematic of cross-regulation of NRC-H by NRC-S, showing redundancy of helper proteins.

reconstruction of the evolution of the superclade, because sugar beet is not an asterid and kiwifruit branches very early in this clade. Thus the authors hypothesise that these species most closely represent the ancestral form that gave rise to the NRC superclade, which in any case must have arisen from a single *NRC* progenitor or indeed coupled *NRC-S* and *NRC-H* genes. They hypothesise that the NRC superclade duplicated and expanded from such an ancestral pair to form the complex genetic structure that is in evidence today (Figure 1).

So our dancers are performing an intricate quadrille, but the steps remain hidden. In the classical mechanism of NLR pairs, the sensor NLR holds the helper NLR in an inactive conformation. Release of this negative regulation after effector perception allows the helper to activate defences. Wu et al propose a slightly different mechanism for NRCs. This model relies on positive activation of NRC-H by NRC-S, rather than suppression of autoactivity. They reason that this allows expansion of the sensor family and their ability to interact redundantly with different helpers. It is an important point because sensor R proteins are highly polymorphic due to positive selection by pathogen pressure. Redundancy would free NRC-S proteins to evolve to recognise changeable effectors without activating NRC-H by loss of negative regulation. As yet there are no data for or against direct interaction of NLR-S and – H proteins. Importantly, *NRC1* was discovered in a screen for genes required for the function of Cf4, an extracellular receptor protein that is not an NLR [11]. No NRC-S component is known in this system, yet a positive role for NRC-H would fit nicely into this pathway. NRC-H genes appear to be under different selection pressures to NRC-S genes, perhaps explaining their relative lack of proliferation and diversification. On the other hand, perhaps this level of expansion is sufficient to accommodate the greater diversification of the sensors – so that they can all still interact functionally with at least one helper. Wu et al suggest that NRC-H evolution may be constrained by their requirement for multiple *R* pathways, an idea that could be tested by examining evidence for purifying selection in this family. It further follows that the functionality of some *R* genes may be restricted taxonomically by a requirement for specific NLR helpers that may be present in limited range of related plant species. Lastly, it is puzzling that NRC-H proteins have not yet emerged as hubs targeted by pathogen effectors, as this would appear an efficient means to shut down multiple recognition pathways. How active NLR couples work in lock step to condition

resistance remains a fascinating area for discovery of new steps in these convoluted dances.

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