

Plant Autoimmunity: When Good Things Go Bad

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A recent study finds that the *Arabidopsis* DM1 and DM2d proteins physically interact and trigger autoimmunity in plants. The DM1–DM2d interaction pattern differs from that of known immune receptor pairs, portraying the versatility in NLR functioning.

Hybrid necrosis is the phenomenon in which a cross between two fit parents yields unfit progeny showing symptoms of permanent activation of stress responses, such as dwarfism, tissue necrosis and sometimes even lethality [1]. Recent studies in the model plant *Arabidopsis thaliana* identified two loci that, when combined, trigger hybrid necrosis: *DANGEROUS MIX 1* (*DM1*) and *DM2d* [2]. Whereas the parental plants (Uk-3 and Uk-1) are perfectly normal, their F1 hybrids carrying *DM1* and *DM2d* are not (Figure 1A). Both loci were previously shown to encode nucleotide-binding leucine-rich repeat (NLR) proteins [3]. NLRs form one of the largest and most variable protein families in plants. Many NLRs function as intracellular receptors, perceiving the presence of pathogen-derived effector proteins. Recognition of the effector activates the NLR, enabling it to trigger immunity, which is often accompanied by cell death [3]. *DM1* and *DM2d* encode NLRs of the so-called TNL sub-group as they carry an amino-terminal toll/interleukin-1 receptor (TIR) domain [2,4]. The mechanistic basis by which these NLRs cause hybrid necrosis was unknown and is the focus of the paper by Tran *et al.*, published recently in *Current Biology* [5].

The necrotic phenotype resulting from co-expression of *DM1* and *DM2d* in *planta* was found to be dependent on typical downstream TNL immune signalling components, such as EDS1 and members of the HSP90–SGT1–RAR1 chaperone complex [6]. Hence, the hybrid necrosis phenotype is genetically indistinguishable from that of an autoimmune response induced by dysregulated immune receptors [7]. To assess whether the DM proteins indeed mimic activated immune receptors, and thereby trigger an immune response,

including local cell death, specific point mutations were made in important functional domains. The P-loop motif is the most conserved motif in NLR proteins and is required for ATP/ADP binding and NLR activity [8]. Mutation of the conserved lysine in the P-loop of either *DM1* or *DM2d* abolished cell death. Subsequent interaction studies demonstrated that *DM1* and *DM2d* are able to form homomers and heteromers through their TIR domains, and to form high order complexes in *planta*. Disturbing the interaction between *DM1* and *DM2d*, by domain swaps or mutations targeting putative TIR interaction interfaces, abolished their ability to trigger cell death. Notably, *DM1* was also found to interact with *DM2g*, a ‘non-dangerous’ paralog of *DM2d*. However, this interaction did not induce cell death, showing that an interaction between *DM1* and a *DM2* member is required – but not sufficient – for triggering autoimmunity. Competition assays with non-functional variants of *DM1* or *DM2d*, which can still form heteromers, revealed an unequal contribution of *DM1* and *DM2d* to trigger autoimmunity. Increasing amounts of inactive *DM1*, but not inactive *DM2d*, negatively affected cell death. These results imply that *DM1* transduces a *DM2d*-generated signal, triggering the induction of immunity and concomitant cell death. Taken together these data show that the *DM1* and *DM2d* pair, by forming a heteromeric NLR complex, triggers a genuine immune response in the absence of a pathogen.

The notion of NLRs working as pairs is a relatively new concept in plant pathology [3,9]. Only recently, examples of pairs have been reported in which a ‘sensor’ NLR is responsible for pathogen perception and an ‘executor’ or ‘helper’

NLR is responsible for activation of downstream signalling. Two generic models are emerging: In the first (e.g. the sensor–executor model) the two NLRs are encoded by one genetic locus and the encoded proteins physically interact. In this case, the sensor NLR carries an integrated domain that mimics the effector target and aids pathogen perception [10]. Effector perception by the sensor NLR relieves the suppression of the executor NLR, unleashing the signalling potential of the latter [11]. Examples of such pairs are found in rice (*RGA5/RGA4*) and *Arabidopsis* (*RRS1/RPS4*). In both cases, (over) expression of the executor NLR alone (*RGA4* or *RPS4*) triggers an autoimmune response, underscoring its capacity to signal independently [12–14]. In addition, the P-loop of the sensors (*RGA5* or *RRS1*) were found to be defective, which beautifully confirms that these NLRs rely on the signalling potential of the executor NLR. The second model (e.g. the sensor–helper model) is based on genetic studies that identified helper NLRs functioning downstream of multiple sensor NLRs. In these cases, the sensor and executor NLR genes are typically encoded by different loci and a physical interaction between the proteins has yet to be shown. Downstream helper NLRs have been identified in several plant species, including the Solanaceae (e.g. NRCs [15,16] and NRG1 [17]) and *Arabidopsis* (*ADR1* [18]). In the latter model, a functional P-loop in the sensor is required and the helper merely requires activation by the sensor NLR, rather than de-repression, as (over)expression of a helper NLR alone does not trigger immune signalling.

Interestingly, the *DM1* and *DM2d* pair does not seem to perfectly fit either model and actually harbours aspects of both.

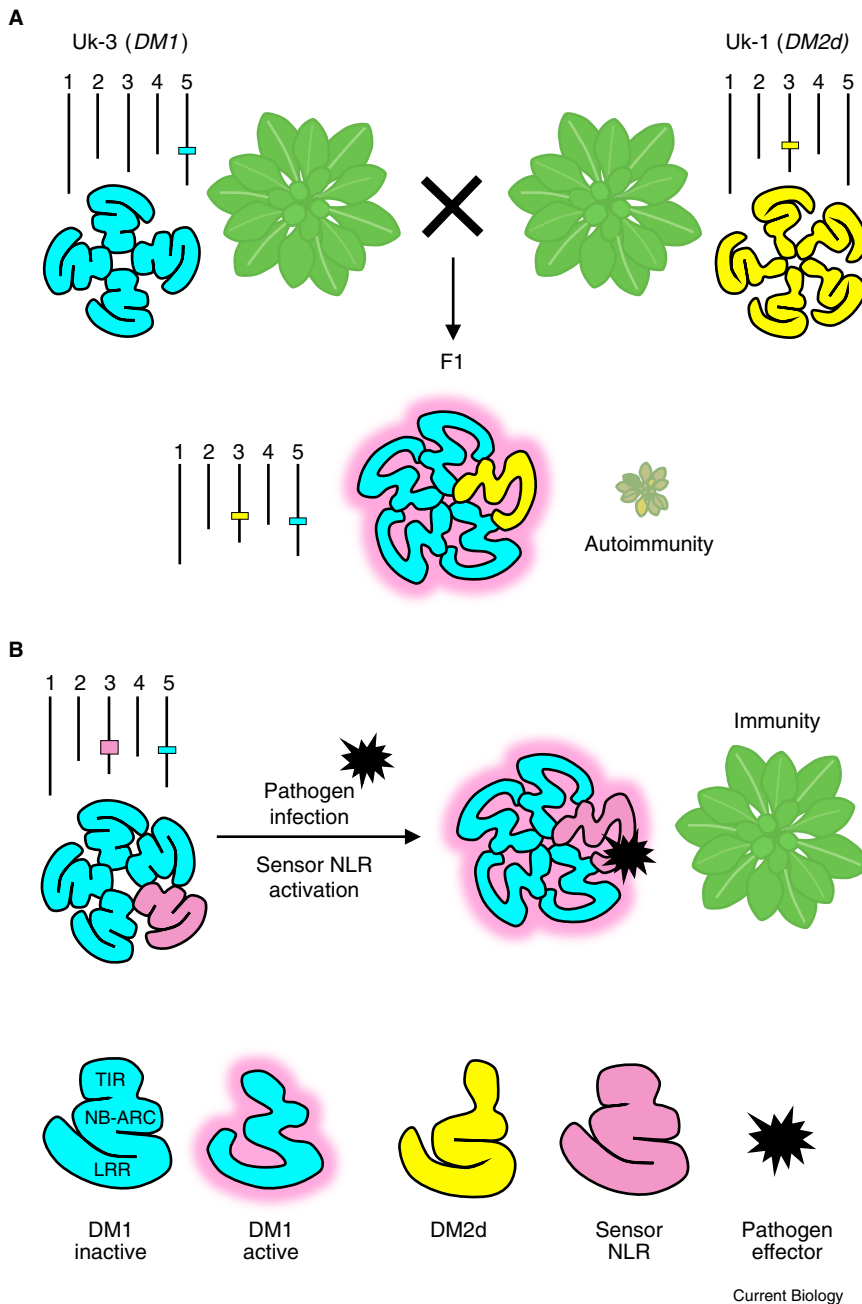


Figure 1. Hypothetical model for DM1- and DM2d-induced autoimmunity.

(A) Phenotypes of the Uk-3 (*DM1*) and Uk-1 (*DM2d*) parents and their F1 progeny (*DM1/DM2d*) and the hypothetical structures of DM protein complexes. *DM1* (blue) maps on chromosome 5 of Uk-3 [2]. *DM2d* (yellow) is a member of the *RPP1* cluster and maps on chromosome 3 of Uk-1. The *RPP1* cluster contains several NLRs (pink) that confer resistance to the oomycete *Hyaloperonospora arabidopsidis* [4]. Interaction of *DM2d* with *DM1* results in formation of a heteromeric complex and immune signalling. Based on the molecular mass of the complex [5] the number of DM proteins is predicted to range between two and five. (B) A model for *DM1*-mediated immunity is proposed in which *DM1* functions as transducer for *DM2d*-like proteins (such as the *RPP1* homologs, pink) that are activated following effector perception.

Like in the sensor–executor model, *DM1* and *DM2d* physically interact, forming heteromeric complexes. However, like in the sensor–helper model both NLRs

require a functional P-loop to induce immunity and both genes are encoded by distinct loci. Furthermore, overexpression of either *DM1* or *DM2d* alone did not

induce immunity, implying that *DM* signalling is based on transactivation rather than de-repression.

Taken together, the *DM1*–*DM2d* study seems to imply a third model for paired NLR-mediated immune signalling (e.g. a sensor–transducer model). In this case, *DM2d* could mimic an erroneous activated sensor NLR triggering *DM1* activation, resulting in autoimmunity. In the endogenous Uk-1 background such autoimmunity is not triggered by *DM2d*, as no functional *DM1* homolog appears to be present at the syntenic locus [2]. In the Uk-3 background, which carries *DM1*, also no autoimmunity is triggered as *DM2d* is not present. Notably, *DM2* homologs are present at the *RPP1* locus, which might encode the genuine sensors for *DM1*. Indeed, *DM1* was found to be able to heteromerize with at least two other members of the *DM2/RPP1* family, one of which has been demonstrated to confer resistance against the oomycete *Hyaloperonospora arabidopsidis* [4]. So, in the absence of *DM2d*, *DM1* might transduce signals from genuine activated sensor NLRs upon pathogen perception (Figure 1B). It would be interesting to investigate which sensors do depend on *DM1* for signalling.

The increasing number and the diversity of NLRs working in pairs suggests that there is a myriad of ways in which NLRs can trigger immunity in plants. Interestingly, cooperating NLRs that function together to combat pathogens are also found in metazoans. A clear example is the NAIP–NLRC4 inflammasome, in which NAIP perceives the pathogen and subsequently forms heteromers with NLRC4 to initiate defence signalling [19]. The notion that plant NLRs might often function in pairs could be one of the explanations why NLR-based resistances are typically not transferable between species [20]. Co-transfer of a complete NLR pair could overcome this limitation. Indeed, the transfer of the RRS1–RPS4 NLR pair from *Arabidopsis* to rapeseed, tobacco and tomato enabled these plants to mount resistance responses to the bacterial pathogen *Pseudomonas syringae*. Transfer of the same NLR pair to cucumber provided resistance to the fungus *Colletotrichum orbiculare* [20]. From this perspective, hybrid necrosis, which often forms a barrier for gene transfer in resistance breeding, might be a

blessing in disguise. In the long run, it might allow identification of NLR pairs functioning together, enabling their transfer to other species. Besides the impact on plant breeding, these studies greatly increase our fundamental knowledge on the versatility and functioning of NLR pairs in immunity.

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