

Inspirational Decoys: a new hunt for effector targets

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A commentary on: **Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread** by Kroj et al. (2016). *New Phytologist*.

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Understanding recognition mechanisms of pathogenic microbes by plants is not only pivotal to crop protection programs, but has also revealed fascinating examples of co-evolutionary biology. A new phase has been initiated with a series of exciting discoveries on ‘integrated decoys’. In this issue of *New Phytologist*, Thomas Kroj and colleagues (Kroj et al., 2016) show that the integrated decoys are diverse, but common in plants and illustrate that this model predicts novel components in plant immunity.

The co-evolutionary arms race between plants and their pathogens has generated complex molecular features that underlie perception of pathogens. Pathogens employ effectors to suppress the host immune system and gain nutrients. But some plants can recognize some of these effectors and activate effector-triggered immunity (ETI), which is an effective immune response that often involves hypersensitive cell death (HR). Most effector recognition systems involve cytoplasmic Nucleotide-binding (NB) and Leucine-rich Repeat (LRR) or NLR receptor proteins, which recognise specific effectors. The amino-terminus of NLR proteins is variable, commonly consisting of a Toll/interleukin 1 receptor (TIR) or a coiled coil (CC) domain, subclassifying NLRs into TNLs and CNLs, respectively (Jones and Dangl, 2006; Dodds and Rathjen, 2010). Recognition of effectors by NLR proteins can either be direct or indirect. In direct effector recognition, direct interaction between an effector and NLR protein is necessary for recognition (Jones and Dangl, 2006). In indirect effector recognition, modifications of effector targets (a guardee) or mimics of true host targets (a decoy) are sensed by NLR proteins (guards) (Jones and Dangl, 2006; Van der Hoorn and Kamoun, 2008; Dodds and Rathjen 2010).

An emerging intermediary mechanism of effector perception, called the ‘integrated decoy hypothesis’ (Cesari et al., 2014), follows the latest discoveries that NLRs have incorporated additional atypical domains referred to as ‘integrated decoy domains’ (or ‘sensor domains’ (Wu et al., 2015)). These additional atypical domains add the effector-sensory capacity to NLR proteins and are thought to be homologs or mimics of operative or true effector target proteins (Cesari et al., 2014; Nishimura et al., 2015; Wu et al., 2015).

In this issue of *New Phytologist*, Kroj et al. (2016) employed InterPro searches to mine the genomes of 31 plants from the GreenPhyl database for the presence of unusual domains integrated into NLRs. These genome searches resulted in three novel findings. The first finding is that the acquisition of atypical domains in NLRs is very common in both monocots and dicots, and even occur in moss. Kroj and colleagues identified 94 different atypical domains residing in 3.5% of the searched NLRs. However, additional atypical domains may have been overlooked because of limitations of InterPro searches. Most strikingly, some atypical domains have been integrated multiple times at different positions in NLR proteins of both the TNL and CNL subclasses, indicating that these acquisitions are independent and relatively frequent and recent evolutionary events. This includes the repeated, independent acquisition of WRKY domains, protein kinases, and BED zinc finger domains (**Fig. 1**). The second finding is that integrated atypical domains are not restricted to paired NLRs, which are encoded by flanking genes and physically interact to mediate effector recognition. Until recently, only paired NLRs were described to contain atypical domains (Cesari et al., 2014; Nishimura et al., 2015). For instance, the rice RGA4 and RGA5 are paired NLRs that confer recognition of *Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 via an atypical RATX1/HMA domain in RGA5 (Cesari et al., 2013). Likewise, the *Arabidopsis* RRS1 and RPS4 are paired NLRs that confer recognition of *Ralstonia solanacearum* effector PopP2 via the atypical WRKY domain of RRS1 (Le Roux et al., 2015; Sarris et al., 2015). The third finding is that several atypical domains can be present at different locations within a single NLR. At4g12020 of *Arabidopsis*, for example, has integrated two WRKY and two different additional atypical domains (**Fig. 1**). Thus, this genome survey showed that NLRs with integrated atypical domains are common and diverse, and occur throughout the plant kingdom.

Kroj and colleagues also confirmed an important prediction from the integrated decoy model. The integrated decoy model predicts that there must be non-integrated paralogs of the decoy that play a role in immunity or other pathogen-related processes. Kroj and colleagues (2016) confirmed this prediction for a rice NLR containing an N-terminally integrated BED zinc finger domain (BED-NLR). A rice gene that encodes a ZBED protein containing three BED domains was selected for further studies because its transcription levels are correlated with partial rice blast resistance. Importantly, a rice T-DNA insertion mutant lacking ZBED has increased rice blast susceptibility, whereas over expression of ZBED in transgenic rice plants causes reduced rice blast susceptibility (**Fig. 2A**). Therefore, ZBED represents a likely effector target, consistent with a key prediction of the decoy hypothesis.

The discovery for a role of ZBED in plant immunity, inspired from the integrated decoy model, raises three new questions. First, the model implicates that there is an effector that targets BED domains. The positive role of ZBED in immunity suggests that the effector prevents a negative effect on pathogen fitness by suppressing ZBED function. The integrated decoy model also predicts that the effector manipulating ZBED should activate the NLR with the integrated BED domain. This implies that the effector is missing from the used rice blast strain or its recognition is suppressed because this strain

causes disease on the rice line carrying the *BED-NLR* gene. The effector manipulating BED domains remains to be identified.

Second, the model predicts that BED-NLR confers resistance to a rice pathogen. For the described BED-NLR, rice blast isolates remain to be identified that are unable to cause disease unless this BED-NLR is absent (**Fig. 2B**). Theoretically, the NLR with integrated BED could also perceive effectors of other rice pathogens. Indeed, Xa1, another rice NLR carrying an integrated BED domain, confers resistance against bacterial blight (Yoshimura et al., 1998). The integrated decoy model also predicts that the BED domain in the NLR would be essential for the recognition of the BED-manipulating effector but also this remains to be demonstrated (**Fig. 2B**).

Third, the integrated decoy model predicts that the integrated BED domain is a decoy that only functions in effector recognition. There have been suggestions that the ‘integrated decoys’ should rather been called ‘integrated sensors’ (Wu et al., 2015), because ‘integrated decoy’ implies that the decoy domain has no function in the absence of the NLR protein, and that has not been proven at this stage. In fact, it has been argued that because the atypical domains have been integrated only recently, they may still have their original function (Wu et al., 2015). Also in case of the BED-NLR, the role of the BED domain in the absence of the NLR remains to be investigated (**Fig. 2C**). In this case, however, ‘integrated sensor’ is not the appropriate nomenclature since it does not express the notion that the integrated domain mimics functional paralogs of the integrated domain.

The widespread manifestation of atypical domains in NLRs adds a layer of complexity to our understanding of perception and disease resistance mechanisms in plants. The vast majority of the 94 discovered atypical domains remains to be studied further. Most of the NLRs carrying these atypical domains have unknown resistance specificities and also the effectors recognised by these NLRs are undefined. As these integrated atypical domains are supposed mimics of effector targets, we need to look into interactions of plants with those pathogens where these atypical NLRs provide resistance against to identify the effector that is recognized. The detailed analyses of these novel atypical domains will unravel fascinating novel elements in host manipulation and NLR-mediated immunity.

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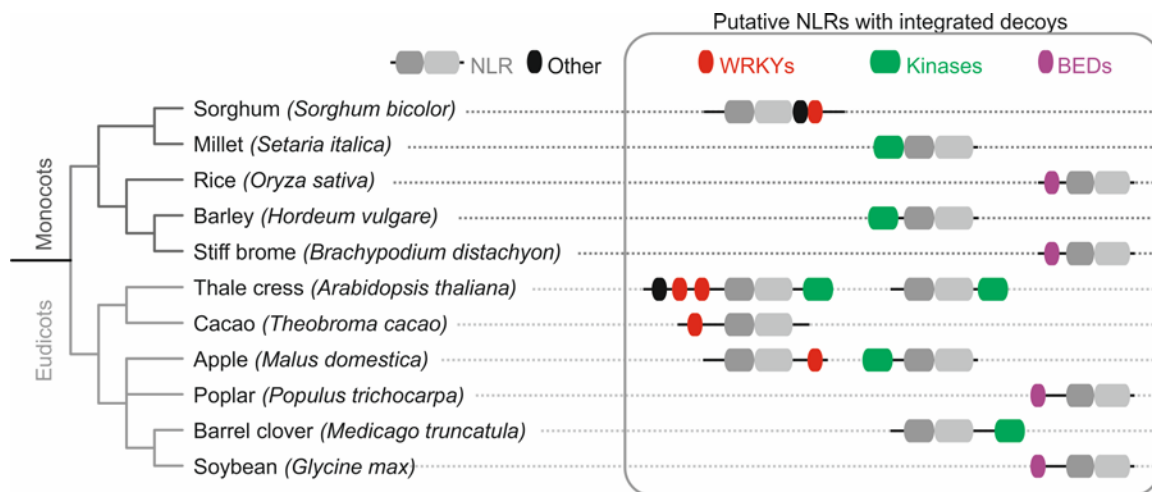


Fig. 1 Independently integrated domains in NLR proteins are common in Angiosperms.

Shown are some of the predicted NLRs that carry one or more additional WRKY transcription factor (red), protein kinase (green), or BED zinc finger (purple) domains. The position relative to the NLR (grey) is indicated. Some WRKY-containing NLRs carry other atypical domains (black). The species tree was generated from the NCBI Taxonomy database using PhytoT.

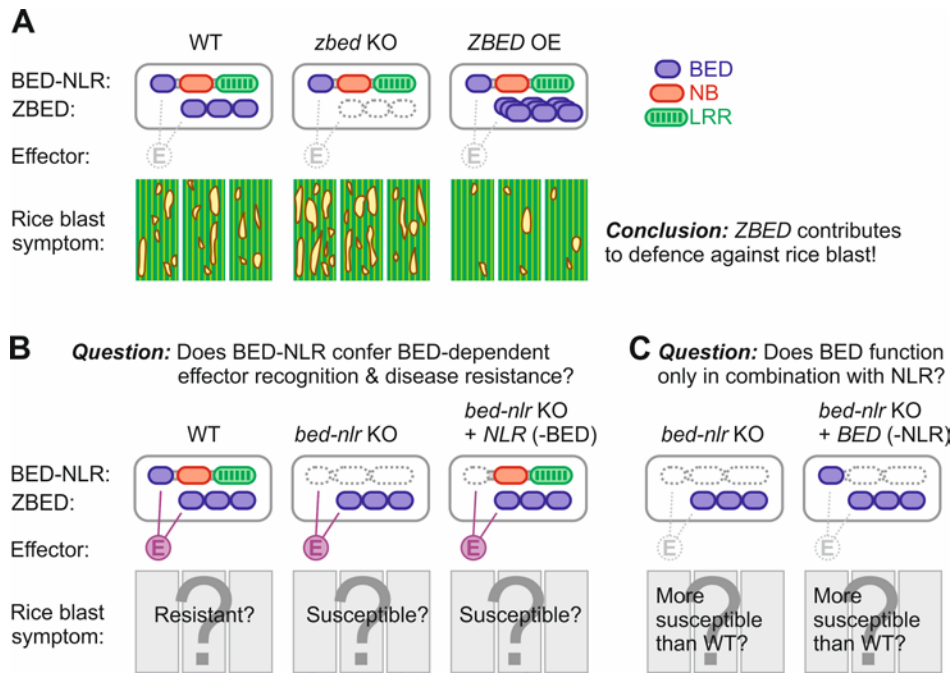


Fig. 2 Infection assays to confirm the predictions of the integrated decoy model.

The integrated decoy model predicts that a protein in rice carrying three BED zinc finger domains (ZBED) is involved in immunity because the BED domain is also integrated in an NLR. **(A)** To test if ZBED acts in immunity, plants lacking (KO, knock-out) or over expressing (OE) ZBED, showed increased and reduced susceptibility for rice blast, respectively, confirming a role of ZBED in immunity. **(B)** The integrated decoy model predicts a role for the BED-NLR pathogen recognition. The model predicts that this NLR detects an effector (E) that manipulates the integrated BED domain. **(C)** A role for the BED domain in the absence of a functional NLR remains to be tested. A true decoy has no function in the absence of the NLR and/or in the absence of the effector.