Oomycetes RXLR Effectors Function as Both Activator and Suppressor of Plant Immunity

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Plant pathogenic oomycetes, such as Phytophthora spp., are the causal agent of the most devastating plant diseases. During infection, these pathogens accomplish parasitic colonization of plants by modulating host defenses through an array of disease effector proteins. These effectors are classified in two classes based on their target sites in the host plant. Apoplastic effectors are secreted into the plant extracellular space, and cytoplasmic effectors are translocated inside the plant cell, through the haustoria that enter inside living host cell. Recent characterization of some oomycete Avr genes showed that they encode effector protein with general modular structure including N-terminal conserved RXLR-DEER motif. More detailed evidences suggest that these AVR effectors are secreted by the pathogenic oomycetes and then translocated into the host plant cell during infection. Recent findings indicated that one of the P. infestans effector, AvrBib2, specifically induces hypersensitive response (HR) in the presence of Solanum bulbocastanum late blight resistance genes Rpi-bib2. On the other hand, another secreted RXLR protein PexRD8 originated from P. infestans suppressed the HCD triggered by the elicitor INF1. In this review, we described recent progress in characterized RXLR effectors in Phytophthora spp. and their dual functions as modulators of host plant immunity.

Keywords: hypersensitive response, Phytophthora, RXLR effector

Plant pathogenic oomycetes: Phytophthora life cycle and infection process

Oomycetes, such as members of the genus Phytophthora, downy mildews and Pythium, are a unique group of fungal-like plant pathogens that cause the most destructive diseases on solanaceous crops, ornamental and other plants (Kamoun and Smart, 2003; Lamour and Kamoun, 2009). Among them, Phytophthora cause one of the most destructive plant diseases in the world. These pathogens are very difficult to control because they are unaffected by the management of fungicides (reviewed by Fry, 2008; Hausbeck and Lamour, 2004; Kamoun and Smart, 2005). The economically important Phytophthora pathogens are Phytophthora infestans on potato and tomato (causes late blight; the cause of the Irish potato famine in 1846), P. sojae on soybean (causes root and stem rot), P. ramorum on oak (causes the sudden oak death), P. capsici on pepper and cucumber (causes Phytophthora blight), and P. palmivora on cocoa etc (cause black pod) (Erwin and Riberiro, 1996; Fry, 2008; Hausbeck and Lamour, 2004; Kamoun and Smart, 2005; Rizzo et al., 2005; Schmitthenner, 1985). Other economically important oomycete pathogens are the downy mildews, cause by Plasmopara viticola on grapevines and Peronospora species (the agent of downy mildew on several crops), which are heterogeneous and the obligate parasites (Hewitt and Pearson, 1999). H. arabidopsis, a natural pathogen of Arabidopsis thaliana, figures prominently in research on disease resistance in Arabidopsis (Allen et al., 2004; Slusarenko and Schlaich, 2003). Altogether, oomycetes cause several billion dollars of damages on crop and ornamental plants annually. The use of fungicides targeted against pathogenic oomycetes can provide some level of disease control. However, the development of crops that possess durable genetic resistance, whereby classical breeding or by genetic engineering, provides the best prospect for effective, economical and environmental sound control of oomycetes disease (Kamoun et al., 1999; Kamoun and Smart, 2005).

Oomycetes, Phytophthora and downy mildews, establish intimate associations with host plants and typically require living host cells to complete their life cycle and infection process known as biotrophy (O’Connell and Panstruga, 2006, Panstruga, 2003). P. infestans establishes a two-step infection style typical of hemibiotrophs. First step, P. infestans requires living host cells for an early infection process that is followed by extensive necrosis of plant tissue resulting in colonization and sporulation on the leaf surface (Kamoun and Smart, 2005). Second step, infected
The RXLR motifs is required for translocation but not for effector activity in plant cells

During infection, oomycetes accomplish parasitic colonization of plants by modulating host defenses through an array of disease effector protein. Oomycetes RXLR proteins, like bacterial effectors of the type III secretion system (TTSS), are modulator protein organized into two main functional domains (Kamoun, 2006). One is characterized by a highly conserved sequence motif at their N-terminus that is the signal peptide and RXLR region, functions in secretion and translocation into plant cell. Other is encoded by the C-terminal region that follows the effector activity (Fig. 1). However, the RXLR motif is not required for effector activity when the deleted RXLR motif is expressed into host cells (Bos et al., 2006; Oh et al., 2009). In fact, deletion analysis of AVR3a and Avrblb2 revealed that the C-terminal 75 amino acids or 34 aa, which excludes the RXLR region was sufficient for avirulence function when expressed directly inside plant cell with cognate R protein (Bos et al., 2006; 2006; Oh et al., 2009).

However, many questions still remained to be elucidated that how does the N-terminal RXLR motif functions in translocation of effectors to inside of host cell. What is the transport machinery of oomycete RXLR effectors? Possibly, transport machinery of RXLR effectors into host cell could be encoded by the oomycetes, the host or/and oomycetes (Birth et al., 2008; Morgan and Kamoun, 2007; Govers and Bouwmeester, 2008; Tyler, 2009). Interestingly, the oomycetes RXLR motif is a similar sequence, like the ‘RXLX(EQ)’ motif, in effectors of the malaria parasite Plasmodium falciparum that invades human erythrocytes (Bhattacharjee et al., 2006). So, is similar machinery used to deliver oomycete and Plasmodium effectors? The answer is “YES”. A few reports demonstrated that the RXLX(EQ) motif is required for translocation of proteins from the parasites Plasmodium into the cytoplasm of host cells, and is similar in sequence, position and function to the oomycete RXLR motif (Bhattacharjee et al., 2006; Govers and Bouwmeester, 2008; Grouttaud et al., 2008; Tyler, 2009). Dou et al. (2008b) examined that the RXLR and dEER motifs, in P. sojae Avr1b effector, can be replaced by the closely related erythrocyte-host targeting domains found in effector proteins of Plasmodium, causes malaria in humans. Also, they demonstrated that RXLR and dEER sequences are both necessary and sufficient to deliver the protein into plant cells. Especially, furthermore, they demonstrated that these motifs function in the absence of the pathogen. Thus, it suggested that pathogen-encoded machinery is not required for effector protein entry into plant cells (Dou et al., 2008b). On the other hand, Whisson et al. (2007) demonstrated that the RXLR motif of P. infestans AVR3a is required for targeting these oomycetes effectors into host plant cells. Furthermore, an AVR3a fluorescent fusion protein was specifically secreted from haustoria and is translocated into the host plant cell in an RXLR-dEER dependent manner (Fig. 2). However, mutated RXLR to AAAA-motif fusion protein translocated in the intercellular spaces (Whisson et al., 2007). In conclusion, it was evidence that the RXLR motif of P. sojae Avr1b and P. infestans AVR3a are needed to confer avirulence on resistant soybean and potato plants when expressed in the pathogen as a host-targeting domain (Dou et al., 2008b; Whisson et al., 2007).
The RXLR effectors are secreted outside the oomycete cell through the general secretory system using endoplasmic reticulum (ER) type signal peptides (SP). And then, the secreted RXLR effectors are transported across a plant-derived membrane, probably the oomycete haustorial membrane, through the ER/Golgi complex, the cytosol, and the extrahusstial membrane. The RXLR effectors are targeted to distinct subcellular compartments within the host cell, thereby crossing two membranes, one from Phytophthora and another from the host. This mechanism of host translocation remains unclear, but may include Phytophthora-encoded translocator protein (Modified from Morgan and Kamoun, 2007).

![Diagram of RXLR effector secretion by Phytophthora](image)

**Oomycetes RXLR effector and plant R gene interaction in Plant**

To date, *P. infestans* delivers effectors to distinct sites of the host cell where they interact with plant targets to reprogram host defense and promote susceptibility (Birch et al., 2006; 2008; 2009; Morgan and Kamoun 2007; Tyler, 2009). Effectors of the first class are secreted into the plant intracellular space (apoplastic effectors, Kamoun, 2006; 2007), whereas class two effectors are translocated inside the plant cell where they targeted to distinct subcellular compartments (cytoplasmic effectors; Kamoun, 2006). One class of cytoplasmic effectors belongs to the RXLR family which contains a highly conserved motif at their N-terminal region that is similar to host-targeting signal. This signal is required for translocation of proteins into the cytoplasm of host cells (Fig. 2, reviewed by Birch et al., 2009; Kamoun, 2007; Morgan and Kamoun 2007). The other hand, the effector activity is encoded by the C-terminal region that follows the RXLR domain (Fig. 2, reviewed by Birch et al., 2009; Kamoun, 2007). A comprehensive knowledge of the structure and function of these various classes of effectors and the perturbations they cause in plants is essential for understanding the molecular basis of the late blight disease (Kamoun, 2006; 2007).

Although oomycetes RXLR effectors are thought to function primarily in virulence, they can also function in innate immunity in plant varieties that carry cognate disease R proteins (reviewed by Kamoun, 2006). Recent findings suggested that oomycete RXLR effectors are specifically induce hypersensitive response (HR) in the presence of the resistance (R) genes in plant varieties (Allen et al., 2004; Armstrong et al., 2005; Bos et al., 2006; Dou et al., 2008a; 2008b; Oh et al., 2009; van Poppel et al., 2009; Vleeshouwers et al., 2008). These effectors are expected to have an avirulence (Avr) activity, thereby activating directly or indirectly HR cell death and associated disease resistance responses mediated by specific R proteins (Fig. 1 and 2, Allen et al., 2004; Armstrong et al., 2005; Bos et al., 2006; Dou et al., 2008a; 2008b; Oh et al., 2009; van Poppel et al., 2009; Vleeshouwers et al., 2008). The “gene-for-gene hypothesis” was introduced by Flor in the 1940s, a well-characterized perception mechanism, is based on plant R products confer recognition of cognate pathogen Avr proteins, and a number of Avr-R gene combinations have since been characterized (Dangl and Jones, 2001). Recent reports demonstrated that secreted effectors from *Phytophthora* that contain a RXLR motif are translocated inside plant cells where they target distinct subcellular compartments (Bhattacharjee et al., 2006; Grouflaud et al., 2008). Effector
activity is thought to be recognized by intracellular R-protein in plant cells (cytoplasmic proteins), culminating in defense signaling and resistance to these pathogens (Kamoun 2006; 2007). These proteins have a nucleotide binding site (NBS) and leucine-rich repeats (LRR) and are called NBS-LRR proteins that are located in plant cytoplasm (Jones and Dangl, 2006).

Some effectors suppress plant immunity and elicitor-induced hypersensitive response

The functions by which RXLR effectors promote virulence are currently under intense study. To date, the virulence activities of oomycete RXLR effectors remain largely unknown, but some cases have been shown to suppress plant immunity such as PAMP (pathogen-associated molecular pattern)-triggered immunity. Recently, Bos et al. (2006) demonstrated that expression of AVR3a could suppress the HR cell death of N. benthamiana triggered by recognition of the oomycete PAMP, elicitin (Fig. 3, so-called INF1, a sterol carrier protein secreted by all Phytophthora spp, Vleeshouwers et al., 2006). Overexpression of the P. sojae RXLR effector Avr1b confers increased pathogen virulence on compatible soybean plants (Dou et al., 2008a), and could suppress the mouse pro-apoptotic protein BAX-triggered PCD (programmed cell death) in N. benthamiana (Dou et al., 2008a). BAX is a key regulator of PCD in animals (Borner, 2003). More recently, Oh et al. found that some Phytophthora RXLR effectors could partially suppress the BAX-induced PCD in N. benthamiana (unpublished data). Likewise, some bacterial effectors can suppress BAX-triggered plant PCD (Abramovitch et al., 2003; Jamir et al., 2004). So, these results suggest that the RXLR effectors contribute to oomycetes virulence by suppressing defensive PCD in host plants, which is presumably important during the early biotrophic stage of infection by hemibiotrophic pathogenic oomycetes (reviewed by Tyler, 2009). Additionally, H. arabidopsis ATR13 also could suppress callose deposition, production of reactive oxygen species, and PAMP-triggered (by Psedomonas syringae pv. syringae) defense responses in Arabidopsis plants (Jones and Dangl, 2006; Numberger et al., 2004), suggesting that RXLR effectors targets basal defenses against various pathogens (Sohn et al., 2007). In addition, Oh et al. (2009) reported that P. infestans PexRD8 and PexRD36 could partially suppress HR cell death induced by INF1elicitin (Fig. 3). These observations suggested that oomycete RXLR effectors may have acquired a variety of mechanisms of immune suppression.

Conclusion

Over the past decade, more than a dozen late blight R genes were introgressed into potato cultivar from the wild species Solanum demissum, and S. bulbocastanum using classical breeding technique (Ballvora et al., 2002; Fry, 2008). Some of R genes, R1, R3a, RB (Rpi-bhb1) and Rpi-bhb2, cloned from wild potato cultivar (Ballvora et al., 2002; Huang et al., 2005; Song et al., 2003; van der Vossen et al., 2003; 2005). The biology of RXLR effectors is poorly understood but several informative papers have been published recently (Table 1). However, little is known about the corresponding Avr genes from this pathogen so far (Allen et al., 2004; Armstrong et al., 2003; Bos et al., 2006; Dou et al., 2008a; 2008b; Oh et al., 2009; van Poppel et al., 2009; Vleeshouwers et al., 2008). Five sequences of oomycetes genomes were completed or undergoing completion, much information represent a useful opportunity to study the structure-function of RXLR effector as Avr protein in oomycetes-plants R protein interaction. Additionally, comparative transcriptomics and genomics of oomycetes provided tremendous data to our understanding of effector genes regulation, genome organization, and evolution
Table 1. Avirulence RXLR proteins identified in oomycetes of plant pathogen and their corresponding resistance gene.

<table>
<thead>
<tr>
<th>RXLR (AVR) Effector</th>
<th>Pathogen species</th>
<th>Host plant</th>
<th>Subcellular localization</th>
<th>Size (aa)</th>
<th>Signal Peptide &amp; length</th>
<th>RXLR and dEER motif</th>
<th>Corresponding R gene class</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avr1b</td>
<td>P. sojae</td>
<td>Soybean</td>
<td>Cytoplasmic</td>
<td>138</td>
<td>Yes 21 aa</td>
<td>RFLR-EEEDDAGGER</td>
<td>Rps1b</td>
<td>Shan et al. (2004); Doh et al. (2008b)</td>
</tr>
<tr>
<td>Avr2a</td>
<td>P. infestans</td>
<td>Potato</td>
<td>Cytoplasmic</td>
<td>147</td>
<td>Yes 21 aa</td>
<td>RLLR-EENEETSEER</td>
<td>R3a</td>
<td>Armstrong et al. (2005); Bos et al. (2006)</td>
</tr>
<tr>
<td>Avr1bl1</td>
<td>P. infestans</td>
<td>Potato</td>
<td>Cytoplasmic</td>
<td>152</td>
<td>Yes 21 aa</td>
<td>RSLR-DEER</td>
<td>Rpi-blb1 CC-NBS-LRR</td>
<td>Vleeshouwers et al. (2008)</td>
</tr>
<tr>
<td>Avr1bl2</td>
<td>P. infestans</td>
<td>Potato</td>
<td>Cytoplasmic</td>
<td>100</td>
<td>Yes 23 aa</td>
<td>RSLR</td>
<td>Rpl-blb2 CC-NBS-LRR</td>
<td>Oh et al. (2009)</td>
</tr>
<tr>
<td>PiAve4</td>
<td>P. infestans</td>
<td>Potato</td>
<td>Cytoplasmic</td>
<td>287</td>
<td>Yes 24 aa</td>
<td>RFLR-DEKNEER</td>
<td>R4</td>
<td>Poppel et al. (2008, 2009)</td>
</tr>
<tr>
<td>ATR1</td>
<td>H. parasitica</td>
<td>Arabidopsis</td>
<td>Cytoplasmic</td>
<td>311-324</td>
<td>Yes 15 aa</td>
<td>RALR-DDDEER</td>
<td>RPP1 TIR-NBS-LRR</td>
<td>Rehmany et al. (2005)</td>
</tr>
<tr>
<td>Avr1a</td>
<td>P. sojae</td>
<td>Soybean</td>
<td>Cytoplasmic</td>
<td>121</td>
<td>Yes 15 aa</td>
<td>RXLR</td>
<td>Rps1a Unknown</td>
<td>Qutob et al. (2009)</td>
</tr>
<tr>
<td>PiAVR2</td>
<td>P. infestans</td>
<td>Potato</td>
<td>Cytoplasmic</td>
<td></td>
<td></td>
<td></td>
<td>R2 CC-NBS-LRR</td>
<td>Lokossou et al. (2009)</td>
</tr>
</tbody>
</table>

*P. sojae, Phytophthora sojae; P. infestans, Phytophthora infestans; H. parasitica, Hyaloperonospora parasitica

Domain structures of the corresponding resistance gene products are indicated as follows: CC, Coiled-Coil; TIR, Toll-interleukin 1 receptor; NBS, Nucleotide Binding Site; LRR, Leucine-rich repeat.

(Ahns and Kamoun et al., 2009; Jiang et al., 2008; Tyler et al., 2006; Win et al., 2007). In this review, we described the recent findings on a diverse function of the RXLR proteins of oomycetes cytoplasmic effectors for basic understanding of plant-oomycetes interactions.

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References


Judelson, H. S. and Blanco, F. A. (2005). The spores of Phytophth-


Rehmanny, A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong,


