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Receptor-like kinase complexes in plant innate immunity

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A REVIEW ARTICLE

INTRODUCTION

Autotrophs, like plants, are the source of nutrients for heterotrophs. Plants are members of complex communities and have co-evolved commensal and pathological relationships with microbes. A fine balancing act is required to effectively combat invasion by pathogenic heterotrophs while effectively guarding resources for vegetative and reproductive growth (King and Roughgarden, 1982). This entails appropriately timed activation of defense responses to conserve energy for producing numerous healthy progeny, thus increasing evolutionary fitness through this adaptive plasticity (Sultan, 2000). Detecting harmful heterotrophs and converting this recognition to intracellular signals aimed at combating the intruder and alerting surrounding tissue, is a major challenge, especially since pathogens co-evolve with their hosts to evade discovery (Frank, 1992; Lehti-Shiu et al., 2009).

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FIGURE 1 | Complexes of Xa21, FLS2, and EFR. (A) A model to summarize the current data regarding Xa21 function as discussed in this manuscript. (B) Illustration of the complexes formed by the RLK-FLS2. The yellow dots indicate phosphorylation of a protein. Yellow arrows indicate phosphorylation of a substrate protein. Yellow blunt arrows indicate dephosphorylation of a substrate protein. Green dots and green arrows indicate ubiquitination. Black arrows indicate translocation, association or dissociation. (C) Selected interactors of the RLK EFR. (D) Shows biological effects of selected RLK activation.

e et al., 2010a) and the RD kinase BAK1 (Chinchilla et al., 2007; Heese et al., 2007). These receptors present the core of our current knowledge regarding RLKs involved in defense.

Xa21

The Xa21 extracellular domain is composed of 23 LRRs and was one of the first eukaryotic RLKs found to be involved in resistance (Song et al., 1995; Wang et al., 2006). Xa21 binds the Xanthomonas oryzae pv. oryzae (Xoo) secreted tyrosine (Tyr) O-sulfonation peptide AxYS22 (Lee et al., 2009). Much has been learned about the function of Xa21. For example, the amino acids Ser686, Thr688, and Ser689 in the cytosolic JM domain are important for stability and for endoplasmic reticulum (ER) processing (Xu et al., 2006; Park et al., 2010a). Phosphorylation of residues in the JM domain is also critical for the activation of Xa21 and binding of at least four Xa21-binding proteins named XB3, XB15, XB24, and XB10 (OsWRKY62; Park et al., 2010b) associated with Xa21 via the JM domain. These interactions are all dependent on Thr705 since mutation of this JM domain residue abolishes XB-Xa21 binding (Chen et al., 2010a).

XB3 is an E3 ligase important for Xa21 accumulation and is a substrate for Xa21 kinase activity, although the biological relevance of this relationship is still unclear. After Xa21 binds AxYS22, XB3 is activated by transphosphorylation and likely leads to cleavage of a negative regulator of defense or even of itself, allowing other interactions to take place (Wang et al., 2006).

Xa21 is regulated by two proteins through phosphorylation; XB15, a protein phosphatase 2C (PP2C) and XB24, a protein with intrinsic ATPase activity (Park et al., 2008). XB15 dephosphorylates Xa21 and XB15 over-expression reduces Xoo resistance while xb15 null-mutants exhibit increased cell death and resistance to Xoo. This would point to a negative regulatory role of XB15. On the other hand, XB24 promotes autophosphorylation of Xa21 and may be required to prevent proteolytic cleavage of Xa21. The complex between XB24 and Xa21 dissociates upon Xoo infection or AxYS22 binding (Chen et al., 2010b). Phosphorylation, especially in the JM domain, plays a critical role in Xa21 stability. It is clear that autophosphorylation of certain residues in Xa21 promotes an inactive state but the exact changes in phosphorylation status upon pathogen infection remain largely unknown.

Xa21 binds to the WRKY transcription factor XB10 and this binding requires an active Xa21 kinase domain. Binding of the AxYS22 peptide to Xa21 leads to translocation of a Xa21 kinase domain-GFP fragment to the nucleus where it interacts with XB10.
AxYS22 ligand is perceived, the majority of the receptor is found
www.frontiersin.org
August 2012 | Volume 3 | Article 209 | 3
flagellin-derived peptide flg22 has been shown using125I-labeled
DC3000 (Gómez-Gómez and Boller, 2000). Direct binding of the
pathogens including conserved protein flagellin from a broad class of bacterial plant
The FLS2 (flagellin sensing 2) receptor recognizes the well-
Greeff et al. Receptor -like kinases
interacting partners (Seo et al., 2011). Although the biological
scale yeast two-hybrid study revealed yet another set of Xa21
interacting components (Lu et al., 2010). BIK1’s role in PTI is dependent
complex interactions with major immune response regulators
and may thus provide RLK signaling complexes with the ability
to discriminate between biotrophic and necrotrophic pathogens
(Laluk et al., 2011). Importantly, bik1 mutants display enhanced
susceptibility to Pto DC3000, reduced flg22 responsiveness, as well
as compromised flg22-induced resistance to virulent Pto DC3000.
The BIK1-related kinase, PBS-like kinase 1 (PRL1) and PRL2 also
interact with FLS2 and BAK1. pbl1 mutants show less reduction in
PTI responses but the effect seems to be additive to BIK1 function
(Zhang et al., 2010).

BAK1, BIK1, SERK1, and SERK2 have also been shown to interact
with BIK1 (BAK1-interacting receptor-like kinase 1), an
active protein kinase. The bir1 mutant exhibits increased resistance
to biotrophic P DC3000 and Hyaloperonospora arabidopsidis
Noco2, due to apparent R protein activation (Wang et al., 2011).
The bir1 phenotype is partially rescued in bir1 pud4 double
mutants, and is completely rescued in the bir1 pud4 bso1 (suppress-
or of bir1-1) triple mutant. Phytoalexin deficient 4 (PAD4) is one
of the critical components required for Toll/interleukin-1 receptor
(TIR) R protein signaling. Many constitutively active defense phe-
notypes that result from activated TIR R proteins are suppressed
by PAD4 loss of function (Wiemer et al., 2005; Palma et al., 2010;
Zhang et al., 2012). The aforementioned results thus indicate that
the bir1 phenotype is partly dependent upon R protein activation,
although the majority of defense induction in bir1 occurs through
SOBIR1. SOBIR1 is also a R, and over-expression of SOBIR1
leads to activation of cell death. SOBIR1 does not function in
flg22 sensing and does not interact with BIR1. Exactly how loss
of BIR1 activates SOBIR1 is a mystery (Gao et al., 2009), and it
is still uncertain whether BIR1 has a role in the PAMP signaling
pathway.

Kinase-associated protein phosphatase (KAPP) interacts with
the FLS2 kinase domain (Gómez-Gómez and Boller, 2000), and
this interaction may be important for receptor endocytosis upon
activation as was found for ASERK1 (Shah et al., 2002). KAPP
has also been found in complexes with other RLKs (Williams
et al., 1997; Stone et al., 1998) but whether it functions as a
general regulator of a broader spectrum of RLKs needs to be
explored.

FLS2 also interacts with E3 ligases that polyubiquitinate the
receptor after flg22 signaling. FLS2 is subsequently degraded
by the proteasome, which might constitute a mechanism for
attenuation as has been described for the mammalian Toll-like
receptor 4 (TLR4) and TLR9 (Chuang and Ulevitch, 2004).
Plant U-box 12 (PUB12) and PUB13, both E3 ubiquitin lig-
ases, have been shown to be BAK1 phosphorylation targets,
and this modification is required for their association with
FLS2. This phosphorylation is reminiscent of the previously
mentioned Xa21 phosphorylation of XB3. PUB12 and PUB13 con-
trol flg22-dependent, proteasome-mediated degradation of FLS2
The nuclear translocation is important for Xoo resistance and
the Xa21 kinase domain/XB10 complex probably affects defense
gene expression (Park and Ronald, 2012). Whether this or a simi-
lar mechanism also applies to other RLKs is currently unknown,
but future studies will likely address this issue. Recently, a large-
scale yeast two-hybrid study revealed yet another set of Xa21
interacting partners (Soo et al., 2011). Although the biological
significance of these discoveries in signaling remains to be seen,
they may provide interesting clues to the functions of Xa21 and
other RLKs.

To help proteins fold properly, the ER contains a number of
chaperones including BiPs (binding immunoglobulin protein)
that bind N-glycosylated proteins and direct them to the ER
(Melchior and Helenius, 2000). Xa21 is also N-glycosylated and
interacts with BiP3, an HSP70-like ATPase located in the ER,
and this is important for correct folding and functioning of the protein
(Park et al., 2010a). While a pool of Xa21 locates to the PM where
AtXa21 ligand is perceived, the majority of the receptor is found
in the ER.

AflS2

The FLS2 (flagellin sensing 2) receptor recognizes the well-
conserved protein flagellin from a broad class of bacterial plant
pathogens including Pseudomonas syringae pv. tomato (Pto)
DC3000 (Gómez-Gómez and Boller, 2000). Direct binding of the
flagellin-derived peptide flg22 has been shown using125I-labeled
peptides (Chinchilla et al., 2006), but a recent report also ampli-
cates FLS2 in unstimulated Xoo Ax21 peptide perception. These
two peptides are not sequence related, which makes the finding
quite astonishing (Danna et al., 2011).

FLS2 was recently shown to form homo-dimers independently of
flg22 binding, but whether this dimerization is important for
receptor function is not known (Sun et al., 2012). However,
it is well-established that FLS2 forms heterodimers with BR1-
associated kinase 1 (BAK1) (Chinchilla et al., 2007; Schulze et al.,
2010) in the presence of bound flg22. BAK1 is a common com-
ponent in many RLK signaling complexes, and was first identified for
its requirement in brassinosteroid signaling via the receptor
BAK1 (Li et al., 2002). The essential role of BAK1 in flg22 sensing
was revealed by the marked reduction of flg22-induced responses
in bak1 mutant plants (Chinchilla et al., 2007; Hesse et al., 2007).
Importantly, the BAK1–FLS2 interaction most likely does not compete
with other known BAK1 interactors such as BR1, and the BAK1–
FLS2 interaction is therefore not responsible for BR-mediated
PAMP defense suppression (Albrecht et al., 2012). BAK1 is a mem-
ber of the somatic embryogenesis receptor kinase (SERK) family
comprising 5 members, SERK1, SERK2, BAK1/SERK3, BAK1-
like (BBK1)/SERK4, and SERK5. FLS2 interactions with SERK1,
SERK2, SERK5, and BBK have been detected, but its predomi-
nant association is with BAK1. BAK1 and BKK1 are thought to
act cooperatively in PAMP signaling and resistance to biotrophic
pathogens (Roux et al., 2011). BAK1 and FLS2 also interact with
Botrytis-induced kinase 1 (BIK1), which is a receptor-like cytoplasmic kinase (RLCK)
implicated in resistance to necrotrophic pathogens (Veronese et al.,
2006). BAK1 and FLS2 phosphorylate BIK1 (Lu et al., 2010) and
BIK1 in turn phosphorylates both FLS2 and BAK1. This is thought


www.frontiersin.org
August 2012 | Volume 3 | Article 209 | 3
"fpls-03-00209" — 2012/8/22 — 19:04 — pag e3—# 3
Despite being a transmembrane protein, FLS2 does not depend critically on N-glycosylation for its function as has been found for EFR (Nekrasov et al., 2009; Sujo et al., 2009; Häweker et al., 2010). However, FLS2 has recently been shown to interact with the reticulon-like proteins RTNLB1 and RTNLB2. RTNLB1/2 are together involved in regulating FLS2 transport from the ER to the plasma membrane (Lee et al., 2011). In addition, stomatal cytokinesis defective 1 (SCD1) was identified by mass spectrometry as an FLS2 interaction partner. Scdl mutants display SA-dependent enhanced resistance to infection with Pto DC3000, as well as enhanced accumulation of PR1 transcripts and hydrogen peroxide. However, the same mutants are less sensitive to PAMPs, with reduced seedling growth inhibition and ROS production in response to flg22 or elf18 (Koraisik et al., 2010).

**EF-Tu RECEPTOR**

EF-Tu receptor is a LRR-RLK that recognizes the peptide elf18 from bacterial elongation factor (EF)-Tu. EFR and BAK1 have also been shown to interact in a ligand-dependent manner (Roux et al., 2011). Indeed, many of the signaling components downstream of FLS2 and EFR and FLS2 are shared. While both EFR and FLS2 are capable of associating with all members of the SERK family, BKK1, SERK1, SERK2 have a stronger association with EFR than with FLS2 (Roux et al., 2011). This might allow EFR to avoid pathogen effector action on the single SERKs. Studies of SERK function have been difficult due to their apparent redundancy and the lethality of some double mutants such as serk1 serk2, bak1-4 bkk1-1, and bak1-5 bkk1, enabled study of non-lethal double mutants. This revealed that BAK1 and BKK1 act cooperatively in regulating FLS2 transport from the ER to the plasma membrane (Lee et al., 2011). In rice, RTNLB1/2 are together involved in regulating FLS2 transport from the ER to the plasma membrane (Lee et al., 2011). In rice, RTNLB1/2 are together involved in regulating FLS2 transport from the ER to the plasma membrane (Lee et al., 2011).

**LyM FAMILY**

Chitin elicitor receptor kinase 1 (CERK1) is the best studied Arabidopsis LyM-RLK (Kaku et al., 2006; Miyas et al., 2007; Wan et al., 2008), and direct binding of chitin to CERK1 has been detected (Iizasa et al., 2010; Petutschnig et al., 2010). Unlike FLS2 and EFR, CERK1’s perception of fungal chitin is RAK1-independent. In rice, Chitin elicitor-binding protein (CeBIP), a LyM domain-containing protein, associates with OsCerk1 and these proteins function together in a hetero-oligomer receptor complex to elicit chitin signaling in a ligand-dependent manner (Shimizu et al., 2010). Two LyM domain proteins, LYM1 and LYM3, have recently been shown to be important for peptidoglycan (PGN), but not chitin recognition. LYM1 and LYM3 are not functionally redundant, and it has been proposed that LYM1, LYM3 and CERK1 may form a complex or complexes. cerk1 is hypersusceptible to Pto DC3000 and shows reduced sensitivity to PGN, pheno-copying lym1/lym3, however CERK1 does not bind to PGN. Further, given the fact that neither LYM1 nor LYM3 contain a cytoplasmic domain, a LYM1/LYM3/CERK1 complex seems likely (Wüllmann et al., 2011). RLKs often hetero-oligomerize for optimal functioning as seen in the co-operativity of FLS2/BAK1, EFR/BAK1 and PEPR1/PEPR2.

**CERK1 FAMILY**

Another RLK, FERONIA (FER) was first shown to control pollen tube reception (Escobar-Restrepo et al., 2007). However, the expression of FER throughout the plant suggests a general function not strictly associated with root development or pollen tube reception. Indeed, FER has more recently been shown to aid powdery mildew (PM) penetration into host cells (Kessler et al., 2010) and to be responsible for susceptibility to the oomycete H. arabidopsidis (Nibau and Cheung, 2011). It is suspected that FER might play a role in controlling localization of MLO family proteins, known to be important for PM infection (Corsonetti et al., 2006), as it does for NTA during pollen tube reception. This however still needs to be seen, as well as whether ROS signaling has an effect on MLO localization. Given the many roles of FER it is not surprising to find that it is important for disease resistance as well.

FER appears to exert its signaling functions by controlling ROS production. FER was shown to interact with guanine nucleotide exchange factors (GEFs) that regulate RHO GTPases (RAC/ROPS). RAC/ROP is known to play important roles in stress-induced responses. In rice, the binding of a RAC/ROP called Rac GTPase to NADPH oxidases has been characterized, and Rac GTPase was shown to be required for ROS production (Wong et al., 2007). In Arabidopsis, Rop2 was shown to co-immunoprecipitate with FER. In addition, transgenic plants expressing constitutively active, GTP-bound Rop2 displayed increased ROS production (Cheung and Wu, 2011). This indicates that a FER-GEF-RAC/ROP complex is likely able to affect ROS production. While ROS plays a role in root development, there are hints that FER is involved in ROS production during PAMP signaling in leaves. For example, FER is enriched in detergent-resistant membranes (DRMs) after flg22 treatment, and FER shows flg22-induced phosphorylation (Benschop et al., 2007). FER mutants also exhibit enhanced ROS production, and aberrant stomatal responses upon flg22 treatment (Keinath et al., 2010). The increase...
in ROS production in the fer mutant is puzzling given the reduced Rho GTPase activity in this mutant (Duan et al., 2010). The relationship between FLS2 and FER in the control of ROS production is very interesting and should attract attention in the near future.

CONCLUDING REMARKS

There have been enormous advancements in our knowledge about RLK signaling in the last decade, but many questions still remain unanswered. For example, the link between the FER receptors and production of ROS and activation of MAP kinase is still missing. Nevertheless, a quite comprehensive picture of the route from receptor activation to enhanced defense gene expression has emerged for Xa21 and similar data for FL52 and EFR are sure to come to light.

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www.frontierl.org

August 2012 | Volume 3 | Article 209 | 5
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