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Pattern recognition receptors and their role in plant immunity - a minireview

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Abstract. An essential feature for innate immunity is the ability of organisms to distinguish pathogens in a highly specific manner. In the last decades, in animals and also in plants, pattern-recognition receptors (PRRs) involved in the perception of bacteria and fungi were discovered. They are able to perceive pathogen-associated molecular patterns (PAMPs), molecules that are essential for pathogen survival, very conserved and difficult to modify without function loss. In this article are described three intensive studied PRRs (FLS2, EFR and XA21) and is briefly recalled how they act after activation. There are also mentioned some kinases known as being part of the downstream signalling cascades and how is eventually modulated gene expression in case of an infection.
Key Words: PAMPs, PRRs, FLS2, EFR, XA21, BAK1.

Rezumat. O caracteristică importantă a sistemului imun este capacitatea lui de a recunoaşte organismele patogene într-o manieră foarte specifică. În ultimele decenii s-au descoperit atât la animale, cât şi la plante receptori de recunoaştere a modelului (PRRs) implicaţi în recunoaşterea agenţilor patogeni. Aceşti receptori sunt capabili să recunoască modelele moleculare asociate patogenilor (PAMPs), molecule care sunt esenţiale pentru supravieţuirea lor, sunt foarte conservate şi foarte greu de modificat prin evenimente precum mutaţia, fără ca să fie riscată pierderea funcţiei. În acest articol sunt descrişi trei PRRs intens studiaţi (FLS2, EFR şi XA21) şi modul lor de acţiune. De asemenea, sunt amintite câteva kinaze cunoscute în prezent ca făcând parte din cascadele de semnalizare iniţiate de aceşti receptori, dar şi cum acestea în cele din urmă reuşesc să modifice expresia genică în cazul infecţiilor.
Cuvinte cheie: PAMPs, PRRs, FLS2, EFR, XA21, BAK1.

General notions about pathogen-recognition receptors (PRRs). One of the main challenges for the immune system is discriminating between potential harmful pathogens and self molecules and this objective is achieved due to recognition of molecules that are specific to microbes.

Both plants and animals can recognize conserved molecular patterns that are present at the pathogens surface. They are called pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) and exhibit several features, being conserved molecules - characteristic of a whole class of microbes and indispensable molecules - essential for pathogen survival, difficult to modify without function loss. By detecting PAMPs or injury-related structures that signal danger in the case of wounding and toxin-induced damage, organisms are capable of defence. Host defense mechanism is initiated by pattern recognition receptors (PRRs) (Albert et al 2010; Zipfel 2008).

The first PRR was discovered in 1985 by Christiane Nüsslein-Volhard in *Drosophyla melanogaster* and it was called Toll receptor (Hansson & Edfeldt 2005). In vertebrates exist homologous proteins called Toll-like receptors (TLRs). Even if plants do not present clear homologs with TLRs, they have gene families that encode receptor-like kinases (RLKs) and receptor-like proteins (RLPs). Both classes of receptors contain an extracellular domain (ECD), a single-pass transmembrane (TM) domain, but RLPs lack an intracellular domain, present in RLKs, where it has a precise signalling function. They belong to gene families composed of more than 600 genes in *Arabidopsis* sp., respectively 1000 in rice, and only for a few of them their roles in development, growth

or defence were assigned (Monaghan & Zipfel 2012; Zipfel & Felix 2005). According to ECD structure, PRRs can be divided in two categories: 1) leucine-rich repeat domain (PRR) - usually implicated in protein-protein interaction, in the case of TLRs and the one discussed now in PAMPs binding; 2) lysin motifs (LysM), that usually recognize and bind peptidoglycan (PGN) and structurally related molecules but also detect fungal chitin. LysM, in combination with a TM domain and a Ser/Thr kinase domain, is a unique structure found only in plants (Zhang et al 2007; Segonzac & Zipfel 2011).

Perception of PAMPs by PRRs culminates with immune response and elimination of infection. Because PRRs contain a single TM domain, it is unlikely that after ligand binding at ECD, the information can be passed "vertically" through the receptor. It is probable that this event is followed by homo- or heteromerisation of the receptors, and this leads to activation of signalling pathways through their kinase intracellular domain (Albert et al 2010). The perception of PAMPs initiates the first layer of plant innate immune system called PAMP-triggered immunity (PTI). In their attempt to avoid PTI, microorganisms secrete virulence factors (effectors) which are injected into the host cells. As a complementary mechanism, plants own resistance genes (R-genes) whose transcription products (R-proteins) are able to recognize effectors effect (Segonzac & Zipfel 2011).

Effector-triggered immunity (ETI) is now started, which has a more powerful and rapid effect, in general culminating with local cell death. Controlled cell death in animals is promoted by caspase, but plants lack this protein. A recent research shows that a β-subunit of the 26S eukaryotic proteasome is responsible for caspase-3-like activity expressed by *Arabidopsis thaliana* in case of infection with *Pseudomonas syringae* pv. *tomato*. In this situation, antibacterial factors and programmed cell death-promoting signals are released from the vacuole, leading to apoptosis. Also, in *A. thaliana*, programmed cell death can be triggered by an R-protein via METACASPASE 1 (AtMC1) (Spoel & Dong 2012).

Examples of PRRs

Flagellin sensing 2 (FLS2). Flagellin is the key component of the prokaryotic flagellum, being a globular protein that arranges itself in a hollow cylinder. N- and C-termini are conserved and form the inner core of the protein, while the central portion is found at the surface and is more variable. Plants recognize flg22 epitope, a conserved portion of 22 amino acids (AA) from the N-termini of the protein while mammals detect a specific part of flagellin, domain D1 (Zipfel & Felix 2005).

The receptor for PAMPs is Flagellin sensing 2 (FLS2), which belongs to the protein family Leucine-Rich Repeat Receptor Kinases (LRR-RKs). The exact binding site for flg22 is not known. Studies show that *Arabidopsis* mutated in FLS2 are more susceptible to infections with the bacterium *P. syringae* pv. *tomato* DC3000. Also, some pathogens were successful in evading recognition by FLS2 because they accumulate mutation in flg22. Although PAMPs are under a strong negative selective pressure due to their essential role in microorganisms survival, they are under positive selection pressure in order to evade the host immune system (Zipfel 2008).

FLS2 has a co-receptor, BAK1 [BRI1 (*Botrytis*-induced kinase 1)-associated receptor kinase 1]. It is believed that BAK1 plays an important role in activation of receptor kinases and different intracellular signaling pathways. After flg22 binding, it is assumed that the intracellular domain of FLS2 suffers structural changes, that lead to heterodimerization with the co-receptor BAK1 (Albert & Felix 2010).

In the resting state, FLS2 interacts with BRI1. After flg22 perception, FLS2 associates with BAK1. BAK1 is then activated and phosphorylates BRI1, which in turn transphosphorylates the FLS2/BAK1 complex. BRI1 is now released from the complex and activates downstream signalling pathways. At least two MAPK cascades are initiated, which lead to phosphorylation of the protein MKS1 (the substrate for MPK4) and the transcription factor AtWRKY33. MKS1 is essential for activation of jasmonate (JA)-dependent defense gene expression and also for repression of salicylic acid (SA)-dependent resistance. AtWRKY33 (WRKYGQK sequence at N-terminal domain) is phosphorylated by MPK3/MPK6, which in turn will promote the production of camalexin, an antimicrobial phytoalexin, and therefore will inhibit the growth of pathogenic fungi and

bacteria. AtWRKY33 also appears to be important in combating necrotrophic pathogens through a positive regulation of JA- and ET-mediated defense response signalling (Park et al 2012; Andreasson et al 2005; Zheng et al 2006). KAPP (Kinase-associated protein phosphatase) blocks the activated FLS2 signalling and attenuates the immune response. A model for FLS2-flg22 interaction and the events that are triggered by this can be seen in Figure 1.

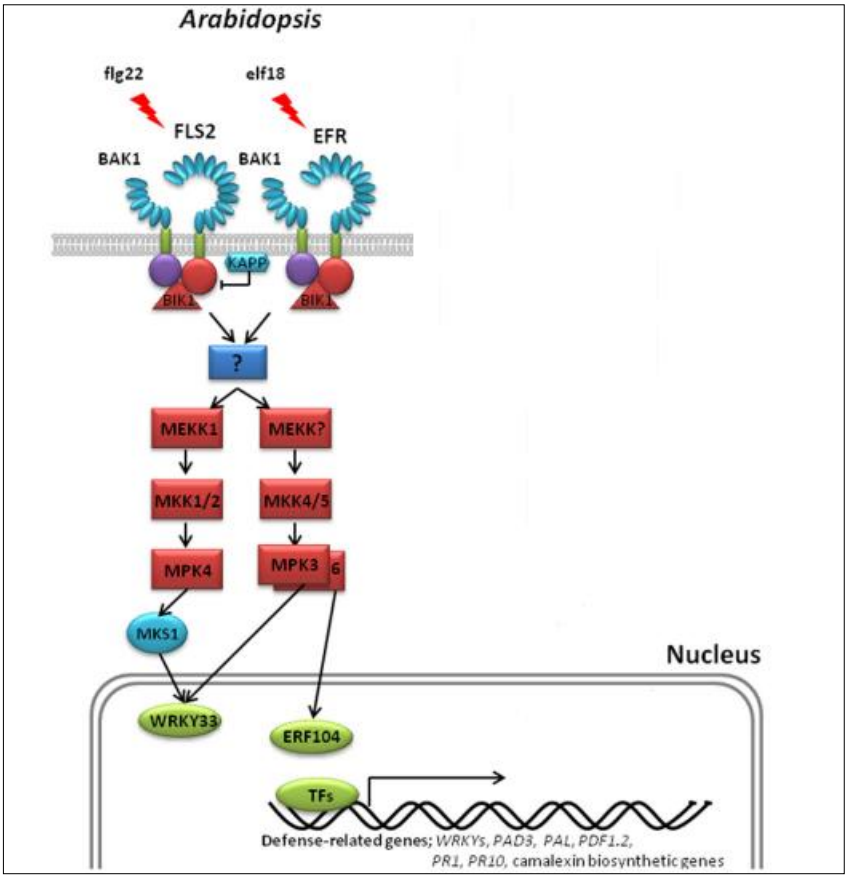

Figure 1. Signalling pathway activated by FLS2-flg22 and EFR-elf18 interaction (Park et al 2012).

Elongation factor Tu receptor (EFR). EFR is a LRR-RLK that recognizes a fragment of 18 AA, motif elf18, which belongs to bacterial elongation factor Tu. This motif contains acetyl-xKxKFxR as a core structure, and it is highly characteristic and unique for EF-Tu from bacteria. This protein binds to all aminoacyl-tRNAs, except fMet-tRNA and selenocysteine-tRNA, and catalyses the incorporation of a new AA into the peptide chain in a GTP-dependent process (Kunze et al 2004).

EF-Tu is essential for protein synthesis in bacteria and it is the most abundant cytoplasmic protein. It possesses all the characteristics for being a PAMP: is highly abundant, conserved and important for microbial survival. I mentioned earlier that EF-Tu is a cytosolic protein, but now arises the question about how EF-Tu becomes visible for the plant. One possibility is that lysis of dying bacteria during plant colonisation should release sufficient EF-Tu to stimulate the receptor, which needs only picomolar concentrations to become activated. Another explanation comes from humans, where was found that EF-Tu from *Pseudomonas aeruginosa* binds to human complement regulator Factor H and plasminogen to evade complement activation. Therefore, it is now believed that EF-Tu is also surface localised, although it lacks any sequence or signal for membrane insertion (Zipfel 2008; Park et al 2012; Postel & Kemmerling 2009).

EFR was described by Kunze et al (2004). In their experiment they use plants mutated in the flagellin receptor gene FLS2 where flg22 treatment has no effect. After they use crude bacterial extract, plants become resistant to following infections. This

suggests that bacterial extracts contain factors, others than flg22, which can trigger plant immunity.

EFR is a close homologous of FLS2, both belonging to the same subclade XII of LRR-RLKs. Since they are structurally similar and have similar functions, it is assumed that more members of clade XII may be receptors for unidentified PAMPs (Postel & Kemmerling 2009).

Even if flagellin and EF-Tu are perceived by two different receptors, the same sets of cellular responses are triggered. Combined treatments with both PAMPs, in doses of 100 nM of the peptides, result in MAPKs activation, without additive effect. This result suggests that these kinases belong to the same cellular pool of enzymes. A model for EF-Tu-elf18 interaction and the events that are triggered by this can also be seen in Figure 1. Still, flg22 induced extracellular alkalinization with a time lag of 30s, but treatment with elf18 lasted 70s. Extracellular alkalinization results from altered ion fluxes across plasma membrane and can serve as a rapid and robust assay in evaluation of PAMPs perception (Zipfel et al 2006; Park et al 2012).

ERF104 (ethylene-responsive transcription factor) appears to be phosphorylated by MPK6, but not by MPK3, and stimulate ethylene (ET) production. ET is often associated with resistance to necrotrophic pathogens, induction of systemic resistance and is often antagonistic with JA or SA. Also, ET-activated transcription factors EIN3 and EIL1 bind to *FLS2* promoter and activate transcription (Zipfel 2013; Bethke et al 2009).

Further, flg22 - FLS2 interaction has been observed to induce disruption of the MPK6/ERF104 complex, and then ERF104 is now able to bind at the promoter PDF1.2 (plant defensin 1.2). According to MedNet Plant pathway Database, PDF1.2 is a small anti-microbial peptide, located in the endomembrane system and cell wall and also is a marker gene for activation of SA and JA signalling. It is known that SA is implicated in SAR occurrence, but JA holds antagonistic action when accumulated in case of infection with necrotrophic pathogens (Park et al 2012; Takahashi et al 2004).

BRI1-associated receptor kinase (BAK1) and its association with multiple PAMPs responses. BRI1 (brassinosteroid insensitive 1) is a leucine-rich repeat (LRR) receptor-like kinase (RLKs) involved in brassinosteroids (BR) perception. ECD of BRI1 consists in a leucine-zipper motif, two pairs of cysteine residues and another sequence composed of 70 AA. The intracellular kinase domain is essential for BR signalling. It is considered that, after BR binding to the ECD, this will induce BRI1 dimerisation and activation of the corresponding kinase. Using yeast two-hybrid screening, it was identified that BRI1 interact with BAK1. Due to this fact, now it is considered that BAK1 and BRI1 have affinity for one another, and will form heterodimers after BR binding. This will stabilize the complex, BRI1-BAK1, and activate their intrinsic kinase activities (Nam & Li 2002).

BAK1 is also implicated in multiple PAMPs responses, including EF-Tu, flagellin or bacterial cold-shock protein. It appears to integrate information received from receptor complexes, converting multiple MAMPs perception into downstream signalling pathways (Shan et al 2008).

Two distinct effectors, AvrPto and AvrPtoB, from *P. syringae*, were found to interfere in multiple MAMP - MAPKs mediated signalling. It was believed that effectors act at MAPKs level, but recently it was shown that the suppression occurs upstream of MAPKKK (MAP kinase kinase kinase). Shan et al (2008) demonstrated that AvrPto and AvrPtoB target BAK1 (using a yeast split-ubiquitin assay), a signalling member of multiple PRRs, and also that mutants in AvrPto were unable to suppress MAPKs.

So, even if BAK1 is not by itself a PRR, it helps multiple PRRs to integrate their messages, and is essential for PTI induction.

XA21. It was identified is rise, in 1970s, because was observed that *Xa21* gene is involved in gaining resistance against *Xanthomonas oryzae pv. oryzae* (Xoo). This receptor is capable to recognize a highly conserved peptide involved in quorum sensing, Ax21 (activator of XA21-mediated immunity). For Ax21-XA21 complex formation, is required tyrosine sulfation (axY^S22) from the N-terminal region of Ax21.

For Ax21 secretion are important 3 proteins, members of type I secretion system: raxA, raxB, and raxC - required for activation of XA21-mediated immunity (the rax genes). Type I is the simplest secretion system, consisting in only 3 proteins: an ABC protein (raxB), a membrane fusion protein (MFP) - in this case raxA, and an outer membrane protein (OMP) or raxC. Xoo mutants in any of these genes loose the ability to trigger XA21-mediated immunity and are unable to secrete Ax21 (Park et al 2010; Lee et al 2006).

Other proteins, such as RaxQ and RaxP, are implicated in 3'-phosphoadenosine 5'-phosphosulfate (PAPS) synthesis. Also, RaxST is a tyrosine sulfotransferase which uses PAPS to transfer a sulfuryl group to Ax21. Two others proteins, RaxH and RaxR, form a two-component regulatory system. RaxH is a histidine kinase with a periplasmic N-terminal sensing domain and a highly conserved cytoplasmic C-terminal histidine kinase domain. RaxR is a regulatory protein which coordinates expression of target genes after RaxH autophosphorylation. A working model for the synthesis, regulation, and function of Ax21 can be seen in Figure 2.

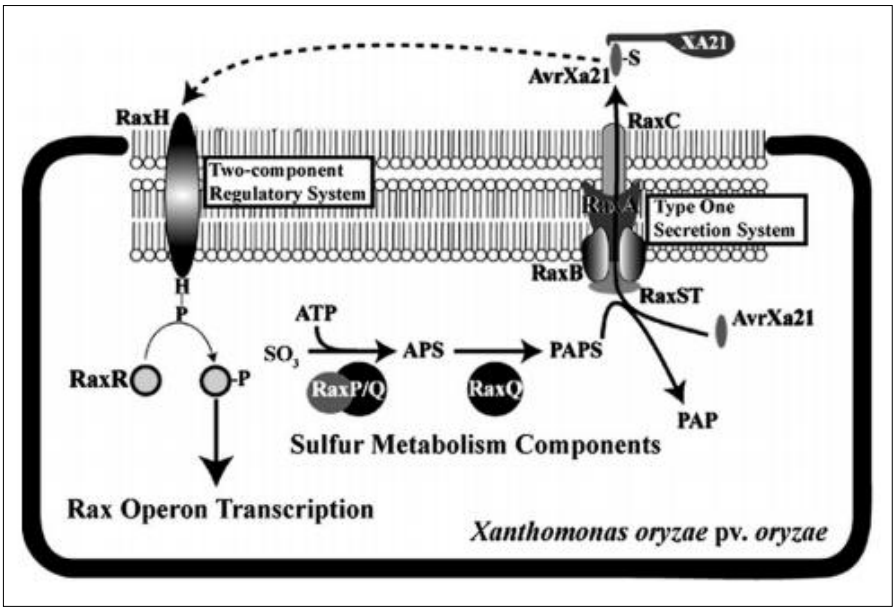

Figure 2. Ax21 synthesis, regulation and function (Lee et al 2006).

As FLS2 and EFR, XA21 is a non-RD kinase, meaning that it carries a cysteine or glycine instead of the arginine at a key location. To function properly, RD kinase (here, I mention just BAK1) needs to associate with non-RD PRRs for transducing the immune response. So, it is belived that XA21 works in correlation with BAK1. After XA21-Ax21 binding, the receptor is autophosphorylated at Thr705. Secondly, Thr705 transfers its phosphoryl group to another XA21 residue, which would activate XA21. It is still unknown if Thr705 autophosphorylation is essential for other non-RD receptor kinases (Park et al 2010).

In animals, after TLRs singnalling begins, non-RD serine/threonine kinase, IRAK1 associates with TRAF6 (tumor necrosis factor receptor associated factor 6) which is a RING (really interesting new gene) ubiquitin ligase. IRAK1 activates then the MAPKs cascade. In plants, XB3 is also a RING, and is structurally and functionally similar with TRAF6. Then, rises the speculation that XA21 acts through XB3, thus activating MAPK cascade MEKK1-MKK4/MKK5-MPK3/MPK6. However, this assumption has not been yet demonstrated (Park et al 2010).

In *Oryza sativa,* WRKY62 (XB10) has been shown to regulate XA21-mediated immune response. Recently, it has been shown that, after XA21-Ax21 interaction, XA21 is cleaved and the intracellular kinase domain contains a nuclear localization signal. In the nucleus, XA21 intracellular domain interacts with WRKY62, and, together, they regulate immune response (Park & Ronald 2012).

Overexpression of WRKY28, WRKY71, WRKY76, and WRKY62 results in activation of PR10 expression, but overexpression of only WRKY62 compromises XA21-mediated immunity by suppressing the transcription of PR1 and PR10, two defense-related genes.

In animals, but also in plants, highly induced immune response can be harmful. For this reason, PRRs signaling are under a strong negative regulation. An important group of negative regulators are protein phosphatase 2Cs (PP2Cs), a group of serine/threonine phosphatases. One protein of this kind is PP2C (XB15), which was identified by yeast-two-hybrid technique using the intracellular fragment of XA21 as bait. XB15 can dephosphorylate XA21 and then regulation by XB15 occurs after Ax21 recognition. In addition, XB24 is an ATPase. XB24 associates with XA21 and promote phosphorylation of certain Ser/Thr sites on XA21, keeping the XA21 protein inactive. This means that regulation by XB24 occurs before Ax21 recognition (Park et al 2010).

XA21 and EFR are highly glycosylated, and this occurs in the ER during maturation. Before membrane incorporation of PRRs, receptors need to fold properly, a mechanism assisted by ER chaperones in a process named ER quality control (ER QC). In plants, members of ER QC are CRT3 (calreticulin 3) and UGGT (UDP-glucose: glycoprotein glycosyltransferase). In addition, for biosynthesis of PRRs are required others ER proteins: SDF2 (stromal-derived factor-2), BiP (heat shock protein 70 - HSP70) and ERdj3B (co-chaperone HSP40). It was observed that overexpression of BiP3 results in compromised XA21-mediated immunity and XA21 degradation via ERAD (ER-associated degradation). Thus, when highly induced immune response occurs, ER stress reacts by targeting the receptor to the ERAD, and thereby preventing possible adverse reactions (Park et al 2010). A model XA21-mediated immunity can be seen in Figure 3.

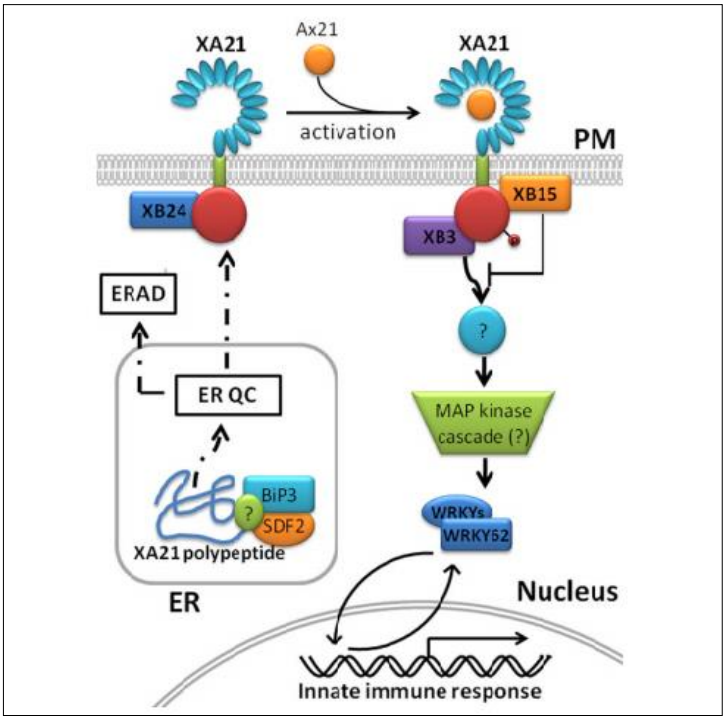

Figure 3. XA21 mediated immunity (Park et al 2010).

Conclusions. For PTI induction, properly recognition of PAMPs must be achieved by PRRs. In plants, these receptors are composed of three parts: ECD, TM and an intracellular domain. In general, they are non-RD kinases which for functioning effectively must be coupled with RD kinase. After PAMPs-PRRs complexes formation, are initiated some signalling cascades, usually involving MAPKs. In the end, transcriptional factors are activated, which will modulate gene expression. Briefly being said, this is how PRRs perform their function.

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