Plant Signaling & Behavior 5:11, 1430-1432; November 2010; © 2010 Landes Bioscience

Chimeric receptors of the *Arabidopsis thaliana* pattern recognition receptors EFR and FLS2

Markus Albert* and Georg Felix
Center for Plant Molecular Biology; University of Tuebingen; Tuebinger, Germany

Key words: pattern recognition receptors, chimeric receptors, MAMP, flagellin perception, FLS2, EFR

Abbreviations: FLS2, flagellin sensing 2; EFR, elongation factor Tu receptor; LRR, leucine-rich repeat; LRR-RK, LRR-receptor kinase; CLV, clavata; Pep25, peptide 25; PEPR, peptide receptor; BRI1, brassinolide insensitive

Submitted: 08/12/10

Accepted: 08/12/10

Previously published online: www.landesbioscience.com/journals/psb/article/13312

DOI: 10.4161/psb.5.11.13312

*Correspondence to: Markus Albert; Email: markus.albert@zmbp.uni-tuebingen.de

Addendum to: Albert M, Jehle AK, Müller K, Lipschis M, Eisele C, Felix G. *Arabidopsis thaliana* pattern recognition receptors for bacterial elongation factor Tu and flagellin can be combined to form functional chimeric receptors. J Biol Chem 2010; 285:19035–42; PMID: 20410299; DOI: 10.1074/jbc.M110.124800.

FLS2 and EFR are pattern recognition receptors in *Arabidopsis thaliana* perceiving the bacterial proteins flagellin and Elongation factor Tu (EF-Tu). Both receptors belong to the >200 membered protein family of Leucine-Rich Repeat Receptor Kinases (LRR-RKs) in Arabidopsis. FLS2 and EFR are engaged in the activation of a common intracellular signal output and they belong to the same subfamily of LRR-RKs, sharing structural features like the intracellular kinase domain and the ectodomain organized in LRRs. On the amino acid sequence level, however, they are only <50% identical even in their kinase domains. In our recently published paper[1] we demonstrated that it is possible to create chimeric receptors of EFR and FLS2 that are fully functional in ligand binding and receptor activation. Chimeric receptors consisting of the complete EFR ectodomain and the FLS2 kinase domain proved to be sensitive to elf18, the minimal peptide required for EF-Tu recognition, similar to the native EFR. In chimeric receptors where parts of the FLS2 ectodomain were swapped into the EFR LRR-domain, the receptor function was strongly affected even in cases with only small fragments exchanged. In this addendum we want to address problems and limits but also possibilities and chances of studying receptor functions using a chimeric approach.

In the Arabidopsis genome exist >600 genes that are predicted to encode for receptor-like kinases (RLKs).[2,3] More than 200 of them have ectodomains with LRRs. Physiological functions have been attributed only to a rather small percentage of them. Examples for known receptor-ligand pairs in *A. thaliana* include the well studied BRI1/Brassionlide,[4,5] AtPEPR1/Pep25,[6] HAESA/IDA[7] or CLV1/CLV3.[8] While these LRR-RKs detect endogenous ligands, other members of this family function as immunoreceptors that detect ligands indicative of 'non-self,' such as pathogen associated molecular patterns (PAMPs). Examples of such LRR-RKs include FLS2 (Flagellin Sensing 2) and EFR (EF-Tu Receptor) from Arabidopsis and XA21 from rice.[9-11] The corresponding ligands have been identified as the flg22-epitope of bacterial flagellin for FLS2, the N-terminus of bacterial EF-Tu represented by the elf18 peptide for EFR, and the sulfated Avr21 peptide from Xanthomonas for XA21, respectively. LRR-ectodomains with related function in pathogen recognition occur also in so-called receptor-like proteins that lack the cytoplasmic kinase domains. Well studied examples include several Cf-receptor proteins which confer resistance against the fungus *Cladosporium fulvum* (Cf) in a gene-for-gene dependent manner. Thereby, different Cf-proteins function as recognition systems with specificity for factors determined by corresponding AvrCf products of the fungal pathogen.[12,13]

Receptor activation of the well studied receptor BRI1 by its ligand brassinolide involves interaction with a further receptor kinase, BAK1 (BRI1-associated receptor kinase 1).[5,14] Most interestingly, BAK1, or one of the four BAK1-related receptor kinases of the SERK protein family, also acts as a co-receptor for the ligand-dependent activation of FLS2, AtPEPR1

Table 1. Functionality of the EFR-FLS2 receptor chimeras in comparison to the native EFR

Receptor	Ethylene response	Oxidative burst	FRK-promoter induction	Binding affinitiy for elf18
EFR	≥0.01 nM	≥0.01 nM	≥0.001 nM	IC_{50} ~10 nM
E-oJM/F	≥0.01 nM	≥0.01 nM	≥0.001 nM	IC_{50} ~10 nM
E-21/F	≥10 nM	≥10 nM	≥0.1 nM	IC_{50} ~10 nM
E-19/F	no response	no response	no response	no binding
F-6/E	no response	≥1,000 nM	no response	IC_{50} ~100 nM

Values indicate the minimal concentrations of elf18 peptide required to trigger significant induction of ethylene synthesis and oxidative burst in leaves of transiently transformed *N. benthamiana* or induction of an FRK-promoter construct in *A. thaliana* protoplasts. The right column shows the relative affinity of the different receptors for the elf18 ligand in competition binding assays; the IC_{50} indicates the concentration of unlabeled elf18 required to compete 50% of radioligand binding.

and EFR.[15-17] It seems that the co-receptor BAK1 plays an important role in activation of receptor kinases, serving different intracellular signaling pathways and output programs.[18]

Up to now, little is known about the molecular details of ligand binding by the ectodomain in the apoplast and how this process leads to activation of the output signaling by the kinase moiety in the cytoplasm. The interaction with the co-receptor BAK1 suggests an activation process involving a ligand-induced intramolecular conformational change of the LRR-RK that then allows heterodimerization with the co-receptor BAK1. An initial task in elucidation of this activation process consists in defining the exact sites in the ectodomains of the receptors that interact with their corresponding ligands. So far, the clearest results for mapping ligand binding sites on LRR-receptor proteins were obtained with directed point mutations within the LRR domains as performed with the tomato receptor-like protein Cf-9,[19,20] and the Arabidopsis FLS2. There, a series of directed point mutations helped to map the LRRs 9–15 as a subdomain essential for interaction with the ligand flg22.[21] Another interesting and promising approach consists in swaps of receptor sub-domains or exchanges of LRRs. In a remarkable, pioneering experiment this approach was used to produce chimeric receptors with the ectodomain of the brassinosteroid receptor BRI1 from Arabidopsis and the kinase domain of the immunoreceptor XA21 from rice.[22] This chimera was reported to recognize the "developmental signal" brassinolide but to trigger characteristic cellular defense responses. In a recent publication[23] a domain swap between the ectodomain of the Wall Associated Kinase 1 (WAK1) and EFR was used to gain evidence for a function of the WAK1 ectodomain as a pectin receptor. Chimeric forms of the Cf receptor-like protein were used to identify subdomains carrying the specificity for the corresponding effectors from the *C. fulvum* pathogens.[24] However, as a limitation of this analysis, for none of these tomato resistance proteins a direct interaction with the corresponding effector proteins of the pathogen could be demonstrated so far.[25]

In our work, recently published in the *Journal of Biochemistry*,[1] we used the *Arabidopsis thaliana* receptors FLS2 and EFR to generate receptor chimeras. The main goal was to study the elf18 binding site in the EFR LRR-domain. In initial attempts we used EFR-constructs lacking some of the LRRs to narrow down the interaction site on the ectodomain. However, all of these truncated ectodomain versions lacking the transmembrane domain or more turned out to be unable in binding elf18 and triggering responses. In a second approach, we used the replacement of receptor parts with fragments from the structurally related receptor AtFLS2. These chimeras were tested for proper expression, localization, functionality in several plant defence related assays and affinity for the ligand elf18 in binding assays. The chimera with the complete EFR ectodomain swapped to the Kinase of FLS2 was fully functional as EF-Tu receptor. Since both receptors are known to trigger the same set of defense responses this might be not unexpected. Nevertheless, it is noteworthy that the two receptors show ~45% sequence identity in their kinase domain, a degree of identity also shared with the kinase domains of receptors involved in other output programs, like BRI1. The 21 LRRs of EFR are sufficient for specifying full affinity for the elf18 as a ligand (**Table 1**). However, replacement of the outer juxtamembrane domain with that of FLS2 leads to a 100-fold reduction in the sensitivity of the receptor for the induction of output responses, without affecting the ligand binding. This is somewhat surprising since these juxtamembrane domains represent a short stretch of ~45 amino acids in EFR and ~40 amino acids in FLS2 that can be thought to exert equivalent functions for EFR and FLS2. Swaps within the LRR-domain resulting in replacement of either the first six (F-6/E) or the last two LRRs (E-19/F) with those of FLS2 completely broke the functionality of the receptor and these chimeras could not trigger any of the output responses tested (oxidative burst, ethylene production or FRK promoter induction, **Table 1**). While swapping the last two LRRs (E-19/F) also completely abolished binding affinity for the elf18 ligand, the construct representing a swap of the first six LRRs still bound the ligand, albeit with ~10-fold reduced affinity. This impaired affinity of the chimeric receptor F-6/E for elf18 indicates a negative influence of the binding process whereas it is not clear if there is a part exchanged which directly interacts with the ligand or if an intramolecular conformation process might be disabled. Besides the marginal similarity of the FLS2 and EFR ectodomain sequences (31% on base of the aa), one of the main difference of EFR and FLS2 is the length: EFR comprises 1,031 aminoacids whereas FLS2 has 1,173. This difference can be clearly linked to the number of LRRs; the longer FLS2 has 28 LRRs and EFR only has

21 LRRs. Nevertheless, although having differences within the LRR-domain and recognizing two different ligands, EFR and FLS2 seem to use a conserved molecular mechanism of receptor activation, including the heterodimerization with BAK1 and the onset of a common output program in the cytoplasm. A loss in the activation of the chimeras might originate from subtle incompatibilities introduced at the swap sites or by exchanges of the LRR-subdomains that are functionally not equivalent. At present, the LRRs that could provide equivalent functions, such as the interaction with the co-receptor BAK1, have not been identified. Mapping these sites with precise swaps of LRRs from FLS2 and EFR is rendered difficult due to the different LRR numbers. The mentioned problems also imply that chimeras of EFR and FLS2 are not perfect to map ligand binding sites, neither for flg22 nor for elf18. Applying this method, it might be helpful to use proteins that are more conserved in their amino acid sequences and share more structural similarities than EFR and FLS2. For example it will be a purpose to use FLS2 homolog from different plant species or EFR relatives from Arabidopsis or from other plants. A check concerning the amino acid number, conserved glycosylation sites, number and structure of LRRs will be very important before creating chimeric receptors.

Although the "fine mapping" of a ligand binding site within a receptor ectodomain seems to be difficult and still needs some optimization, we could show that the approach of "receptor chimearization" works well in principal. The exchange of ectodomains which define specificity for different input signals (elf18 or flg22) resulting in controlling the same output signal has been demonstrated successfully. Altogether, reprogramming in- and output of receptor kinases, as first described by He et al.[22] might be an important tool to investigate and to manipulate plant defence and development.

References

1. Albert M, Jehle AK, Mueller K, Eisele C, Lipschis M, Felix G. *Arabidopsis thaliana* pattern recognition receptors for bacterial elongation factor Tu and flagellin can be combined to form functional chimeric receptors. J Biol Chem 2010; 285:19035-42.
2. Shiu SH, Bleecker AB. Plant receptor-like kinase gene family: diversity, function and signaling. Sci STKE 2001; 113:22.
3. Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KF, Li WH. Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. Plant Cell 2004; 16:1220-34.
4. Li J, Chory J. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell 1997; 90:929-38.
5. Nam KH, Li J. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell 2002; 110:203-12.
6. Huffaker A, Pearce G, Ryan CA. An endogenous peptide signal in Arabidopsis activates components of the innate immune response. Proc Natl Acad Sci USA 2006; 103:10098-103.
7. Stenvik GE, Tandstad NM, Guo Y, Shi CL, Kristiansen W, Holmgren A, et al. The EPIP peptide of INFLORESCENCE DEFICIENT IN ABSCISSION is sufficient to induce abscission in arabidopsis through the receptor-like kinases HAESA and HAESA-LIKE2. Plant Cell 2008; 20:1805-17.
8. Trotochaud AE, Hao T, Wu G, Yang Z, Clark SE. The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. Plant Cell 1999; 11:393-406.
9. Gómez-Gómez L, Boller T. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell 2000; 5:1003-11.
10. Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, et al. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts agrobacterium-mediated transformation. Cell 2006; 125:749-60.
11. Lee SW, Han SW, Sririyanum M, Park CJ, Seo YS, Ronald PC. A type I-secreted, sulfated peptide triggers XA21-mediated innate immunity. Science 2009; 326:850-3.
12. Van der Hoorn RA, Kruijt M, Roth R, Brandwagt BF, Joosten MH, De Wit PJ. Intragenic recombination generated two distinct Cf genes that mediate AVR9 recognition in the natural population of *Lycopersicon pimpinellifolium*. Proc Natl Acad Sci USA 2001; 98:10493-8.
13. Van der Hoorn RA, Roth R, De Wit PJ. Identification of distinct specificity determinants in resistance protein cf-4 allows construction of a cf-9 mutant that confers recognition of avirulence protein avr4. Plant Cell 2001; 13:273-85.
14. Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC. BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell 2002; 110:213-22.
15. Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, et al. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 2007; 448:497-500.
16. Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, et al. The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci USA 2007; 104:12217-22.
17. Schulze B, Mentzel T, Jehle AK, Mueller K, Beeler S, Boller T, et al. Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. J Biol Chem 2010; 285:9444-51.
18. Chinchilla D, Shan L, He P, de Vries S, Kemmerling B. One for all—the receptor-associated kinase BAK1. Trends Plant Sci 2009; 14:535-41.
19. Wulff BB, Chakrabarti A, Jones DA. Recognitional specificity and evolution in the tomato-*Cladosporium fulvum* pathosystem. Mol Plant Microbe Interact 2009; 22:1191-202.
20. Wulff BB, Heese A, Tomlinson-Buhot L, Jones DA, de la Peña M, Jones JD. The major specificity-determining amino acids of the tomato Cf-9 disease resistance protein are at hypervariable solvent-exposed positions in the central leucine-rich repeats. Mol Plant Microbe Interact 2009; 22:1203-13.
21. Dunning FM, Sun W, Jansen KL, Helft L, Bent AF. Identification and mutational analysis of Arabidopsis FLS2 leucine-rich repeat domain residues that contribute to flagellin perception. Plant Cell 2007; 19:3297-313.
22. He Z, Wang ZY, Li J, Zhu Q, Lamb C, Ronald P, et al. Perception of brassinosteroids by the extracellular domain of the receptor kinase BRI1. Science 2000; 288:2360-3.
23. Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. Proc Natl Acad Sci USA 2010; 107:9452-7.
24. Wulff BB, Thomas CM, Smoker M, Grant M, Jones JD. Domain swapping and gene shuffling identify sequences required for induction of an Avr-dependent hypersensitive response by the tomato Cf-4 and Cf-9 proteins. Plant Cell 2001; 13:255-72.
25. van der Hoorn RA, Wulff BB, Rivas S, Durrant MC, van der Ploeg A, de Wit PJ, et al. Structure-function analysis of cf-9, a receptor-like protein with extracytoplasmic leucine-rich repeats. Plant Cell 2005; 17:1000-15.