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β-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants

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ABSTRACT

Plant innate immunity relies in first place on the detection of invading microbes. Thus, plants evolved receptors to sense unique molecules of the microbe, the so called microbe-associated molecular patterns or MAMPs. The best studied fungal MAMP is chitin, an important structural building block of the fungal cell wall. Over the past years several plant receptors for chitin have been characterized as well as different strategies adopted by fungi to evade chitin recognition. Despite its strong activity as an elicitor of plant defense chitin represents only a small percentage of the cell wall of most fungi compared to other complex sugars. β-glucan, the most abundant fungal cell wall polysaccharide, also serves as a MAMP, but the mechanisms of β-glucan perception and signaling in plants are largely unknown. In contrast to that the β-glucan recognition and signaling machineries are well characterized in mammals. The C-type lectin receptor Dectin-1 is a key component of these machineries. In this review we describe valuable knowledge about the existence of at least one β-glucan receptor in plants and about the hindrances in β-glucan research. Additionally we discuss possible future perspectives of glucan research and the possibility to transfer the gathered knowledge from mammalian systems to plants.

1. Introduction

Plants are constantly surrounded by microbes that can be detrimental or beneficial. This situation holds true for the aboveground as well as for the belowground tissue of plants. To protect and defend from microbial invaders or to start the symbiosis program, plants need to detect the presence of a microbe. To do so plants take advantage of the presence of microbe-associated molecular patterns (MAMPs) that can be recognized by pattern recognition receptors (PRRs). In general MAMPs are molecules that are (i) not present in the plant tissue, (ii) crucial for the functioning of the microbial cell and (iii) exposed and thus accessible for the plant PRRs. Bacterial MAMPs which are well characterized include flagellin, a component of the flagellum that acts as a proteinaceous elicitor of defense responses (Felix et al., 1999) and components of the cell wall like lipopolysaccharides (Erbs and Newman, 2003) and peptidoglycan (Gust et al., 2007). Most of the studies on fungal derived elicitors focused on chitin. Even though chitin represents only a rather low percentage of the total cell wall mass (Bowman and Free, 2006), this polymer elicits a strong plant response. In the majority of fungi the most abundant cell wall polysaccharide is β-glucan, a well characterized elicitor in fungus-animal systems (Brown and Gordon, 2003) but also in oomycete-plant systems (Sharp et al., 1984a; Yamaguchi et al., 2000). In spite of this there are compelling reasons why β-glucan remains the stepchild in plant research on elicitor-active fungal cell wall polysaccharides in plant systems. This review gives an overview about past and current advances in this field and future perspectives to unravel the still nebulous field of β-glucan as a MAMP in plants.

2. Chitin as MAMP: the usual suspect

Chitin is an essential structural component that confers rigidity to the fungal cell wall to withstand chemical and physical challenges. Chitin is a homopolymer of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc) residues and is one of the most insoluble compounds in nature. It is hypothesized that the polysaccharide is synthesized at the site of its final deployment via a transmembrane chitin synthase (Fig. 1). The chitin building block uridine diphosphate-N-acetylglucosamine (UDPGlcNAc) is shuttled to the cell membrane, where the chitin synthase transfers N-acetylglucosamine residues donated by the UDPGlcNAc from

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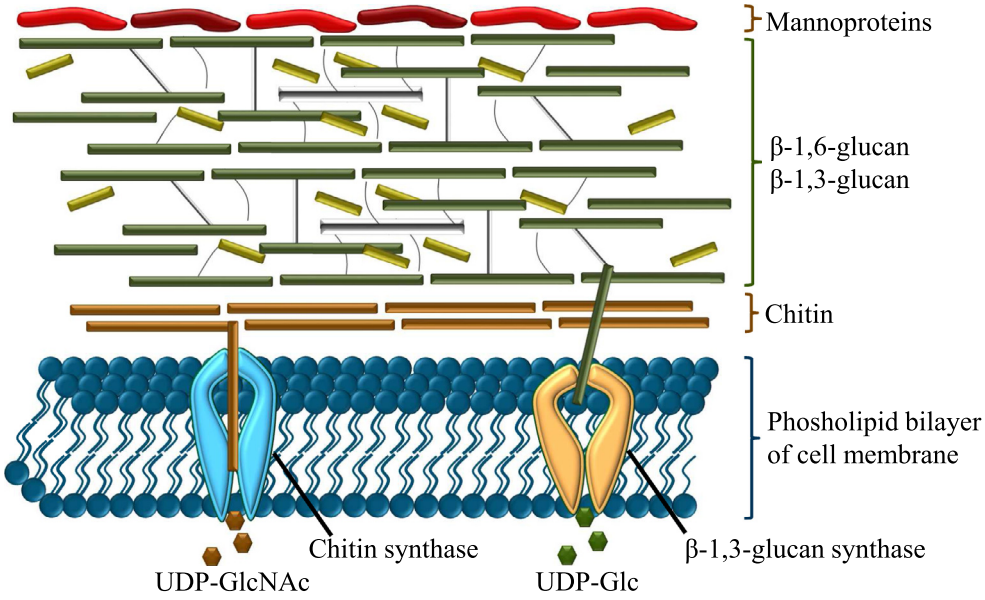

Fig. 1. Schematic overview of fungal cell wall composition. The fungal cell wall mainly consists of chitin (brown) located close to the cell membrane, β-1,3- and β-1,6-glucan (green) adjacent to the chitin fibers and mannoproteins (red) as the outermost part of the cell wall. Chitin is synthesized by transferring N-acetylglucosamine residues from uridine diphosphate-N-acetylglucosamine (UDPGlcNAc; brown hexagon) to a growing fiber that is shuttled through the cell membrane by the transmembrane chitin synthase (light blue). β-1,3-glucan is synthesized by a β-1,3-glucan synthase (yellow) that uses uridine diphosphate-N-glucose (UDPGlc; green hexagon) as a donor to transfer glucose to the extruded β-1,3-glucan fiber.

the cytoplasmic face to the external one to preexisting chitin chains leading to fibril formation (Bowman and Free, 2006). Chitooligosaccharides in their unmodified form with degree of polymerization (DP) 6 to 8 are strong inducers of plant innate immunity. The importance of chitin as a MAMP in plants is emphasized by the fact that in Arabidopsis several PRRs for chitin are present. The first step towards the understanding of chitin signaling in Arabidopsis was made with the discovery of the chitin binding LysM-containing receptor like kinase CERK1 (=LYK1; Miya et al., 2007). Other members of the LYK-family (LysM-containing receptor-like kinases) that act in response to chitin are LYK3, LYK4 and LYK5 (Liang et al., 2013; Wan et al., 2012; Cao et al., 2014). LYK5 was recently shown to physically interact with CERK1 and to have a 200-fold higher affinity to chitooctaose than CERK1. The current model suggests that LYK5 binds to chitin which leads to the formation of a receptor complex with CERK1. This triggers an intracellular signaling cascade mediated by the active kinase domain of CERK1 (Cao et al., 2014). Members of the LYK family are also involved in the perception of acylated chitooligosaccharides with various functional group substitutions on the reducing and nonreducing ends, the so-called lipochitooligosaccharides (LCOs). LCOs are symbiotic signals derived from bacteria (Nod-factors) or from fungi (Myc-LCOs) important in the initiation of the legume–rhizobium and arbuscular mycorrhizal (AM) symbioses respectively (Liang et al., 2014). This raises the question of evolution of chitin perception in plants (Liang et al., 2014). Recent evidence suggests that LCOs perception might have evolved from plant innate immunity signaling (Liang et al., 2014). CEBiP, another LysM-containing receptor originally identified in rice (Kaku et al., 2006), has a homolog in Arabidopsis termed LYM2 (Narusaka et al., 2013). LYM2 is a receptor-like kinase that works independently of CERK1 and triggers different downstream signals, such as plasmodesmata flux regulation (Faulkner et al., 2013). Beside its perception as a MAMP by the plant immune system, chitin is also the target of several plant enzymes that hydrolyze the β-1,4-glycosidic bond between the N-acetylglucosamine subunits to actively attack the fungal invader.

In response to host defense fungi have evolved sophisticated mechanisms to protect their chitin fibrils from hydrolysis and detection. One of the earliest examples of a fungal chitin-binding effector is Avr4 from the tomato fungal pathogen *Cladosporium fulvum*. Avr4 contains a chitin-binding motif first discovered in invertebrates and was shown to protect the fungal cell wall against hydrolysis by plant chitinases accumulating during infection (van den Burg et al., 2006). An additional chitin-binding effector from *C. fulvum*, Ecp6, contains three LysM domains to sequester free chitin from the apoplast to prevent recognition (de Jonge et al., 2010). Crystallographic analysis revealed that having three LysM domains instead of one (like CERK1) enables the formation of a chitin-binding groove with a picomolar binding constant to outcompete the plant receptors (Sánchez-Vallet et al., 2013). Comparative genomic analysis also showed the occurrence of LysM-domain containing effectors in several other plant pathogenic fungi like *Mycosphaerella graminicola* (Marshall et al., 2011) and *Magnaporthe oryzae* (Mentlak et al., 2012), indicating that this could be a widely used mechanism to evade plant immunity. Other strategies of fungi to evade chitin recognition and hydrolysis are either deaminases that convert chitin into chitosan (El Gueddari et al., 2002) or the masking of chitin with α-glucan (Fujikawa et al., 2012). Even though chitin is the best studied fungal MAMP and an important structural component of the fungal cell wall in general it only represents 1–2% of the dry mass of yeast cell walls and 10–20% of the cell walls of filamentous fungi. The most abundant building block of fungal cell walls is indeed β-glucan, which makes up 50–60% of the dry weight. Despite its abundance, studies focusing on the possible role of β-glucan as a fungal MAMP are rare.

3. β-glucan: catch me if you can

Glucans are polysaccharides consisting of glucose units. Two groups of glucans are present in fungi, α-glucans and β-glucans. Most cell wall-bound α-glucans are made of glucosyl units joined by α-1,3-linkages and are water insoluble. Some fungi have

α-1,4-bound glucose units in their cell walls either to interconnect the linear α-1,3-glucan chains or in an alternated way with α-1,3-bound glucose units (named nigeran e.g. in *Aspergillus niger*) (Ruiz-Herrera, 1991). The most abundant β-glucan in the fungal cell wall is β-1,3-glucan which makes up between 65% and 90% of the whole β-glucan content (Bowman and Free, 2006). A large amount of the β-1,3-glucan is covalently bound to β-1,6-glucan in the form of branching polysaccharides (Shahinian and Bussey, 2000). The multibranched β-glucans can be firmly bound to the cell wall or loosely bound and accumulate around the fungus as slime or gelatinous material (Ruel and Joseleau, 1991; Ruiz-Herrera, 2012). The different behavior can be related to the degree of branching of the different glucan polymers or to the molecular size of the polysaccharides. Whereas α-glucans and β-1,3-glucan can be found also in the cell wall of plants, β-1,6-glucan has been found only in the cell wall of fungi and of members of the phylum Chromista, such as in the oomycetes of the genera *Phytophtora* and *Phytium* (Bartnicki-Garcia, 1968; Sietsma et al., 1969), thereby representing a potential MAMP. The synthesis of β-glucan at the fungal cell wall is complex as deduced from the large number of different classes of glucans identified. Several enzymes are involved in this process and although knowledge has advanced rapidly in the last years the mechanism of β-1,6-glucan synthesis remains speculative. As for chitin synthesis a transmembrane enzyme complex directly transfers the newly synthesized β-1,3-glucan chains that can reach up to 1500 glucose molecules into the extracellular space (Douglas, 2001; Ruiz-Herrera, 2012; Fig. 1). β-1,6-glycosidic side branches that account for approximately 3–10% of the total glucan linkages, interconnect the β-1,3-glucan chains to create a rigid network (Bowman and Free, 2006; Latgé, 2007). The connection between the β-glucan and chitin components is often made by β-1,4-linkages. Glucan composition has been analyzed mostly in Ascomycota taxa (e.g. *Saccharomyces cerevisiae*, *Schizzosaccharomyces pombe*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus nidulans*, *Penicillium chrysogenum*, *Monilinia fructigena* and *Candida albicans*), and only few reports exist on the cell wall architecture and composition of Basidiomycota taxa (e.g. *Sclerotium rolfsii*, *Schizophillum commune*, *Armillaria mellea* and *Ustilago maydis*) (Latgé, 2007, 2010; Ruiz-Herrera, 2012). The process of β-glucan synthesis occurs in filamentous fungi at the hyphal tip and at sides of cell growth and branching. Recently Oliveira-Garcia and Deising (2013) showed that in the hemibiotrophic fungus *Colletotrichum graminicola* the expression of the β-1,3-glucan synthase GLS1 is tightly regulated in a temporal and spatial manner during the infection of maize plants. They showed that in appresoria and necrotrophic hyphae the β-1,3-glucan content is massively increased possibly to withstand the high turgor pressure and to ensure high proliferation rates, respectively. In contrast the expression of GLS1 and the β-1,3-glucan content decreased in the biotrophic hyphae, probably to evade an immune response triggered by β-1,3-glucan. In fact, β-1,3 and β-1,4 linked glucan elicitors isolated from filtrates and mycelia extracts of *Colletotrichum* species were shown to be able to induce a response on both compatible and incompatible hosts (Anderson, 1978, 1980). The first articles demonstrating a crucial role for β-glucans as MAMPs in plants were published in the 1970s and 1980s using defense-eliciting heptaglucan structures originating from the cell walls of phytopathogenic oomycetes of the genera *Phytophthora* and *Pythium* (Ayers et al., 1976; Ebel et al., 1976; Albersheim and Valent, 1978; Sharp et al., 1984a,b). In these studies it was shown that β-1,3/-1,6-glucan heptaglucosides are sufficient to elicit phytoalexin production in soybean and that the β-1,6-glycosidic linkage is essential for elicitor activity (Sharp et al., 1984a,b). The ability of plants to respond to glucan elicitors is not universal. Glucans from the fungus *Colletotrichum* are ineffective in the elicitation of phytoalexin production in tomato whereas the glucan elicitors of the oomycetes *Phytophthora megasperma* are only weak elicitors in potato. In 2000 Yamaguchi and colleagues could demonstrate that different β-1,3- and β-1,6-glucan fragments are required to induce phytoalexin biosynthesis in rice cells and in soybean, suggesting that differences in the recognition of glucooligosaccharide elicitor signals exist in these two plants (Yamaguchi et al., 2000). Thus it has been suggested that fungal and oomycete derived β-glucans might serve as recognition cues and non-host resistance at the species level (Albersheim and Valent, 1978; Anderson, 1978). This is in line with the finding that laminarin, a non-defined β-1,3-glucan with β-1,6-glycosidic sidebranches from the marine brown algae *Laminaria digitata* (Chromista Laminariaceae), elicits a wide range of defense responses such as medium alkalization, H_2O_2 production, salicylic acid accumulation and PR gene induction in different plants but not in *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) (Klarzynski et al., 2000; Aziz et al., 2003; Ménard et al., 2004). The linear form laminaripentaose, a synthetic β-1,3-glucan consisting of five glucose units, is reported to be sufficient to trigger the defense responses mentioned before in tobacco, but not in rice (Yamaguchi et al., 2000). Another dimension of β-glucan elicitor research was introduced by the chemical addition of sulfate groups to the laminarin backbone. In 2004 Ménard and colleagues showed that sulfated laminarin (PS3) triggers in tobacco a subset of defense responses including salicylic acid signaling. These responses differ from those triggered by non-sulfated laminarin treatment in tobacco, suggesting the implication of a different receptor (Ménard et al., 2004, 2005). Similar results were obtained for grapevine, where Gauthier et al. (2014) showed that PS3 does not elicit H_2O_2 production and mitogen-activated protein kinase activation but triggered a long lasting membrane depolarization and primed the SA- and ROS-dependent defense pathway upon pathogen attack. Interestingly, in *A. thaliana* ecotype Col-0 it seems that laminarin does not trigger SA-signalling, but Ménard and colleagues demonstrated that PS3 activates PR1-gene expression specifically. This could be a hint for a so far not understood priming effect of PS3 in *A. thaliana*. A comprehensive overview of the plant species, their corresponding β-glucan elicitors and the type of defense response analyzed to date are summarized in Table 1.

In conclusion, the mechanism of action of glucan elicitors is still not fully understood and depends on the plant species and on the origin of the β-glucan elicitor. The structure of the polymers, which have elicitor activity, seems to be fundamental for recognition by the plant immune system. From the presented studies different plant lineages appear to have evolved the ability to respond to microbial glucans displaying a certain degree of variation. This is in contrast to chitin-elicitation, where the mechanism of recognition and its signaling seems to be conserved among the plant kingdom. The difference could be explained by the fact that the structures of the β-glucans of the fungal and oomycete cell walls vary greatly between species. Additionally, β-glucan polymers are also present in the plant cell wall, i.e. as callose (β-1,3-glucan), and it has been proposed that elicitor-active glucan fragments may also originate from plant tissue, possibly functioning as DAMPs (Damage-associated molecular pattern molecules) (Beffa et al., 1996; Klarzynski et al., 2000). Thus the question is, if there is discrimination between the self- and the non-self originating signals in order to mediate downstream defense signaling. Even though there are still open questions, the non-toxicity of laminarin and laminarin-sulfate and their efficiency in stimulating the plant immune system led to the formulation of these polymers as bioprotectant and to the implementation in protection strategies as complementary tool in the form of Vacciplant patented by the french company Goëmar as "agent for stimulation of the natural defences of plants [...]".

Table 1
Overview about plant species, the corresponding β-glucan elicitor and the described defense response.

Plant species	Elicitor fragment	Plant defense response	Reference
Arabidopsis thaliana	Sulfated laminarin	PR1 gene expression	Ménard et al. (2004)
Glycine max	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Sharp et al. (1984b)
Lotus japonicus	β-1,3/-1,6-glucan heptaglucoside	Not tested	Côté et al. (2000)
Lupinus albus	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Cosio et al. (1996)
Medicago sativa	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Cosio et al. (1996)
Medicago truncatula	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Côté et al. (2000)
Nicotiana tabacum	Laminarin	Medium alkalinization[a], ROS burst[a,b], PAL and LOX activation[a], SA accumulation[a], PR-gene expression[a]	Klarzynski et al. (2000)
			Ménard et al. (2004)
	Laminaripentaose	PAL activation	Klarzynski et al. (2000)
	β-1,3/-1,6-glucan heptaglucoside	PAL activation	Klarzynski et al. (2000)
	Sulfated laminarin	ROS burst, electrolyte leakage, scopoletin accumulation, PR-gene expression, SA accumulation, TMV resistance	Ménard et al. (2004)
Oryza sativa	Tetraglycosyl glucitol	Phytoalexin production	Yamaguchi et al. (2000)
	Laminarihexaose	Chitinase induction, PAL activation	Inui et al. (1997)
Phaseolus vulgaris	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Cline et al. (1978)
	β-1,3/-1,4-glucan	Phytoalexin production	Anderson (1978)
	β-1,3/-1,6-triglucoside	Phytoalexin production	Tai et al. (1996)
Pisum sativum	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Cosio et al. (1996)
Solanum tuberosum	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Cline et al. (1978)
Vicia faba	β-1,3/-1,6-glucan heptaglucoside	Phytoalexin production	Cosio et al. (1996)
Vitis vinifera	Laminarin	Medium alkalinization, ROS burst, Ca^{2+}-influx, MAPK activation, defense gene activation, phytoalexin production, hydrolase induction, fungal resistance	Aziz et al. (2003)
	Sulfated laminarin	Membrane depolarization, defense priming	Gauthier et al. (2014)

[a] Klarzynski et al. (2000)
[b] Ménard et al. (2004)

4. Hunting down the elusive plant ß-glucan receptor

An important and crucial step toward advancing glucan research in plants is the identification of plant receptors that bind β-glucans and transduce the signal into the cell. About 10 years after Ayers and colleagues discovered β-glucan to be an elicitor of plant defense, Yoshikawa and colleagues obtained first evidence for a specific receptor in membrane preparations of soybean cells for the binding of laminarin (Yoshikawa et al., 1983). After the discovery that the β-1,3/-1,6-glucan heptaglucoside from the cell wall of *P. megasperma* is a potent elicitor of phytoalexin accumulation in soybean Schmidt and Ebel (1987) identified a "high-affinity binding site" in the membrane fraction of soybean roots. Subsequent studies to characterize the potential receptor revealed a binding affinity of as low as 8 nM (Cosio et al., 1990; Cheong and Hahn, 1991), an approximate size of the receptor of around 70 kDa (Cosio et al., 1992), a potential multimeric nature of the receptor and a minimal structure of the β-glucan elicitor consisting of three β-1,6-linked glucose molecules with two β-1,3-linked glucose sidebranches (Cheong et al., 1991). Further studies identified a glucan-elicitor binding protein (GEBP) from soybean harbouring a glucanase domain and a conserved high-affinity glucan-binding motif, which renders this protein a powerful tool to release and sense elicitor-active fragments from the cell wall of oomycetes (Umemoto et al., 1997; Fliegmann et al., 2004). Building up on these findings in soybean, other legumes were investigated, showing a binding site for the β-1,3/-1,6-glucan heptaglucoside in alfalfa (*Medicago sativa*), bean (*Vicia faba*), lupin (*Lupinus albus*), pea (*Pisum sativum*), *Medicago truncatula* and *Lotus japonicus* (Cosio et al., 1996; Côté et al., 2000). A unifying feature of these studies (comprehensively reviewed by Shibuya and Minami, 2001) is that they all use the β-1,3/-1,6-glucan heptaglucoside liberated from the cell wall of the oomycete *P. megasperma* after acid hydrolysis. During plant–microbe interaction the elicitor-active glucan oligomers are liberated by the activity of plant β-1,3-glucanases (Keen and Yoshikawa, 1983; Ham et al., 1991) and thus the possibility exists that a glucan fragment with stronger elicitor capability is yet to be characterized. In case of an adapted pathogen glucanases activity may lead to the secretion of specific microbe-derived inhibitors that suppress the glucan-induced immune response like it was described for the plant pathogenic oomycete *Phytophthora sojae* (Ham et al., 1997). Additionally to the production of elicitors via hydrolytic activity, Waldmüller and colleagues identified a potent β-glucan elicitor released into the culture medium during the germination of *P. megasperma* spores that also binds to the putative receptor (Waldmüller et al., 1992). Two articles highlight that a cyclic β-1,3/-1,6-glucan, with similar structure to the β-1,3/-1,6-glucan heptaglucoside from *P. megasperma*, is produced in high amounts by the symbiotic bacterium *Bradyrhizobium japonicum*. The cyclic bacterial derived glucan also binds to the potential receptor in soybean (Miller et al., 1994; Mithöfer et al., 1996). Intriguingly, Miller and colleagues found the cyclic β-1,3/-1,6-glucan to be an elicitor of phytoalexin production, whereas Mithöfer and colleagues described it as an inhibitor of phytoalexin production. Additionally, different *Phytophtora* species were also described to secrete water-soluble glucans that suppress cell death, phytoalexin accumulation and β-1,3-glucanase activity in a host specific manner (Henriquez et al., 2012). This contradiction is still unsolved but clearly shows that β-1,3/-1,6-glucans are not only sensed by plants as a danger signal but could also be employed by microbes to suppress plant immunity or to communicate with the plant.

Up to date a putative sequence and domain structure for the plant β-glucan receptor is missing. One reason could be that the most popular ecotype of *A. thaliana*, Col-0, was not shown so far to react to β-glucan treatment with early defense responses

(Ménard et al., 2004). It remains unclear if β-glucan is recognized by any of the *A. thaliana* ecotypes as a MAMP. Additionally, the chemical synthesis of defined β-1,3/-1,6-glucans is challenging (Ning et al., 2003). Thus research is restricted mainly to laminarin from algae and the intensively studied β-glucans of the oomycete genus *Phytophtora* (Robinson and Bostock, 2014). Nevertheless the research carried out with the fungal plant pathogen *Colletotrichum*, the structural similarities between the fungal and the oomycete cell wall and especially the work conducted on fungus-animal systems is pointing towards a role of fungal-derived β-glucans as elicitors of plant defense.

5. Transferring knowledge gathered from mammalian systems onto plants

β-glucans are known as modulators of the immune system in mammals for quite some time. They are described to have anti-inflammatory, anti-infective, anti-tumorigenic and immune-stimulating effects (Czop, 1986; Williams, 1997; Brown and Gordon, 2003). These beneficial effects are connected among others to the ability of β-glucans to activate phagocytosis, reactive oxygen species production and cytokines (Brown et al., 2003; Levitz, 2010). The β-glucan receptor, Dectin-1, was described first in 2000 and identified as the key component for the β-glucan perception and the initiation of an immune response in mice in 2001 (Ariizumi et al., 2000; Brown and Gordon, 2001). The human homolog of Dectin-1 functions in the same way like the murine receptor and is part of a lectin-like gene cluster (Willment et al., 2001; Sobanov et al., 2001). To date Dectin-1 represents the most prominent example of a whole array of β-glucan receptors in animal systems (Brown and Gordon, 2005). Dectin-1 consists of an extracellular C-type lectin domain that is connected to the plasma membrane by a stalk and protrudes to the extracellular space (Fig. 2). The cytoplasmic C-terminus contains an immunoreceptor tyrosine-based activation motif (ITAM) and is devoid of a kinase domain. Dectin-1 is mainly found on the surface of macrophages and to a lesser extent on dendritic cells (Brown et al., 2003). The extracellular lectin domain of Dectin-1 binds to β-1,3-glucans and mixed β-1,3/-1,6-glucans like laminarin, zymosan and complete yeast cells but not to β-1,6-linked glucose subunits alone (Brown et al., 2003; Palma et al., 2006). The minimal length required for binding to Dectin-1 is represented by ten glucose subunits (Palma et al., 2006). Upon binding of a ligand, the intracellular ITAM motif of Dectin-1 gets phosphorylated presumably by SRC, a tyrosine-protein kinase (Brown, 2006). This phosphorylation activates a downstream signaling cascade dependent on spleen tyrosine kinase (SYK) leading to phagocytosis, respiratory burst and cytokine induction (Brown, 2006; Levitz, 2010). Since binding of SYK requires two phosphotyrosines it was proposed that two Dectin-1 receptors form a homodimer upon ligand binding to activate the downstream signaling events (Brown, 2006). This is in line with the crystallographic studies of Dectin-1 with and without ligand present (Brown et al., 2007). Mutational analysis of Dectin-1 identified the amino acids Trp221 and His223 to be crucial for the binding to β-glucan (Adachi et al., 2004) and the crystal structure revealed that these two amino acids are exposed forming a hydrophobic groove that is conserved in the Dectin-1 homologs in mouse, human, chimpanzee, rhesus monkey and cow (Brown et al., 2007). Animal pathogenic fungi evolved several mechanisms like cell wall modification or cell wall shielding to circumvent recognition by Dectin-1 (Sukhithasri et al., 2013).

The question is how much of this knowledge can be transferred from the extensively studied mammalian systems to plant systems? First of all a homolog of Dectin-1 is present in *A. thaliana* and soybean, with an identity of around 30%. In *A. thaliana* the Dectin-1 homolog is the only C-type lectin receptor kinase encoded in the genome (Greeff et al., 2012; Singh and Zimmerli, 2013). The homology mainly corresponds to the C-type lectin domain, whereas it has to be mentioned that the β-glucan binding groove is not conserved. Taking into account that the crystallographic

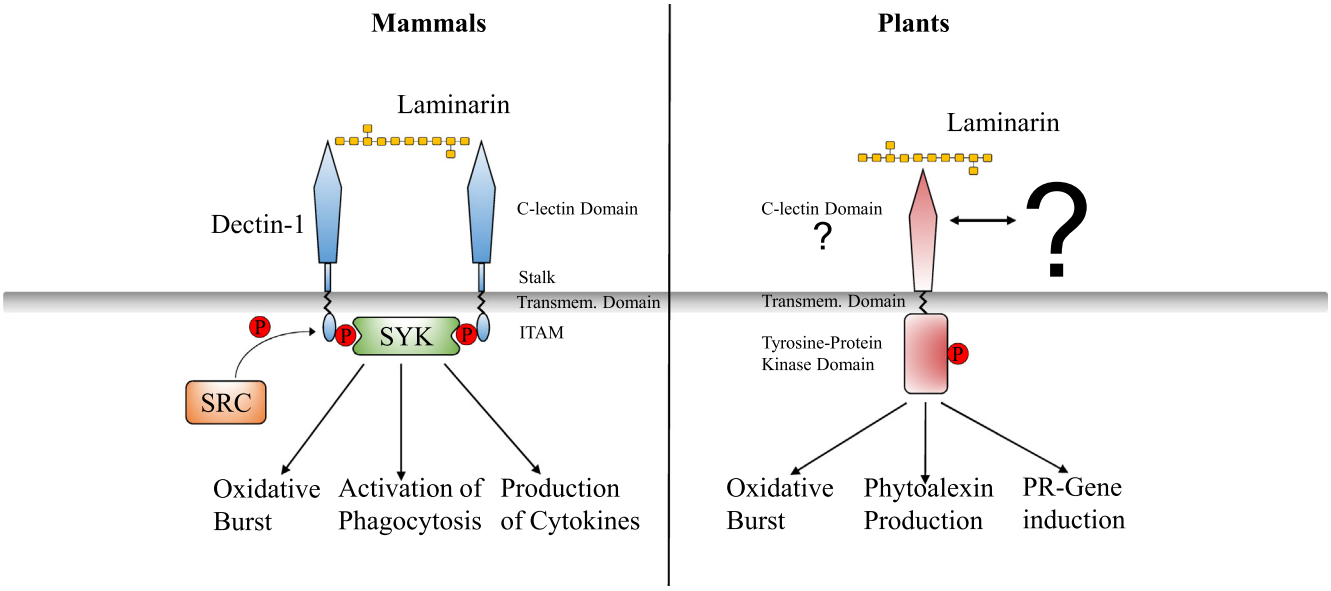

Fig. 2. Transferring existing knowledge from the mammalian β-glucan perception machinery onto plant systems. Recognition of β-glucan in mammals is mediated by Dectin-1. Upon laminarin binding it is proposed that two Dectin-1 proteins form a receptor complex. The intracellular ITAM domain (immunoreceptor tyrosine-based activation motif) of both receptor molecules is subsequently phosphorylated by SRC. Both phosphorylated ITAM domains are necessary to activate the tyrosine-protein kinase SYK (spleen tyrosine kinase) that triggers downstream events like an oxidative burst, the activation of phagocytosis and the production of cytokines. On the plant side not much is known about the β-glucan perception. In plants, upon glucan recognition, H_2O_2 and phytoalexins are produced and PR-genes are activated. The receptor is unknown but a receptor like kinase with 30% similarity to the animal Dectin-1 is present in *A. thaliana* and soybean. The most similar protein to Dectin-1 of *A. thaliana* and soybean contains an intracellular tyrosine-protein kinase domain and thus probably does not require the recruitment of an additional kinase. If this potential receptors of *A. thaliana* and soybean form a homodimer or a complex with another receptor is also not known but seems to be probable.

analysis of Dectin-1 were done using a trimer of β-1,3-linked glucose units, it is possible that different amino acids than those suggested by Brown et al. (2007) are responsible for the binding of longer and branched oligosaccharides such as laminarin. Additionally there is strong genetic evidence that selection pressure from animal pathogens can lead to establishment of polymorphisms in some of the glycan-binding receptors of the innate immune system that result in amino acid substitutions (Taylor and Drickamer, 2014). Recent studies on the human C-type glycan-binding receptor Langerin, which mediates carbohydrate-dependent uptake of pathogens, revealed that a commonly occurring single nucleotide polymorphism (SNP) in the carbohydrate binding site abolishes binding to oligosaccharides with terminal $6SO_4$-Gal and enhances binding to oligosaccharides with terminal GlcNAc residues (Feinberg et al., 2013). Thus, it is plausible that different *A. thaliana* ecotypes recognize different β-glucans. As often found in plants, the putative Dectin-1 receptor homologs in Arabidopsis (Accession number: NM_104110) and soybean (Accession number: XP_003534958) contain an intracellular kinase domain, namely a tyrosine-protein kinase domain like SYK. The fact that Dectin-1 is forming a homodimer upon ligand binding strongly reminds of the mechanism of chitin perception in Arabidopsis (Liu et al., 2012). As mentioned before *A. thaliana* Col-0 was not shown so far to react to laminarin with the production of ROS and Ca^{2+}-influx (Ménard et al., 2004). Despite this, it was reported that PR1 expression is induced in Col-0 by the stimulation with sulfated laminarin (Ménard et al., 2004). Obviously *A. thaliana* Col-0 is lacking some component of the β-glucan perception machinery compared to other plant species or the receptor requires a different β-glucan structure than laminarin for efficient binding and activation of downstream signaling. One possible approach to identify this component makes use of the recently launched 1001 genomes project that collects the genome sequences of just as much Arabidopsis ecotypes (Weigel and Mott, 2009). In this way it could be possible to screen a phenotype (for example H_2O_2 production after β-glucan elicitation) and match that with a given gene/receptor variant (loss or mutation of a β-glucan signaling component). Another way to identify the glucan receptor(s) would be the use of another plant model system. Interestingly the Dectin-1 homolog in soybean has a predicted size of 61 kDa and with possible glycosylation (N-glycosylation sites are predicted in the C-type lectin domain) would fit in size to the 70 kDa high-affinity glucan-binding protein discovered in the early 1990s. However, β-glucan binding might not be restricted to C-Type lectin folds and plants could have evolved different lectin-like receptors for this polymer. In this case the use of a biotinylated glucan ligand to pull down the receptor complex is conceivable, like it was done as a proof of concept for CEBiP and fls2 in rice (Shinya et al., 2010). This approach is unfortunately complicated by the lack of knowledge about the exact structure and the availability of chemically defined β-glucan ligands.

6. Conclusion

Taken together research on fungal derived β-glucan recognition and signaling in plants remains a black box since most of the knowledge was gathered from oomycete-plant or fungus-animal systems. Most plants recognize β-glucan as a MAMP, but a conserved minimal structure that is required for defense elicitation in all plant families has not been yet reported. There are several hints for the presence of a specific β-glucan receptor in legumes but a conclusive candidate has not been described yet. Additionally, the search for a β-glucan receptor is greatly hindered by the fact that *A. thaliana* Col-0 seems not to respond to laminarin as a MAMP. Unfortunately most of the available mutant lines are made in Col-0 background, thus the power of *A. thaliana* as a genetically tractable model system cannot be used to identify a plant β-glucan receptor. One possibility might be the search of the receptor in different *A. thaliana* ecotypes and the use of the Arabidopsis 1001 genome database as bioinformatic foundation to identify the mutated or missing component in *A. thaliana* Col-0. Another option that is suitable in other plant systems is the identification/characterization of the receptor protein using affinity cross-linking with biotinylated glucan ligands. There are hints that at least a homolog of Dectin-1 exists in some plant species. If we also include knowledge about the different mechanisms of chitin perception in plants it is valid to assume that probably there is more than just one receptor involved in β-glucan perception. In general receptor like kinases seem to represent a universal mechanism in plants to perceive microbial signals to trigger an immune response (Boller and Felix, 2009). One aspect that has only briefly been mentioned is the cyclic glucan of the symbiotic bacterium *B. japonicum* that also binds to the potential receptor in soybean and may act as a symbiotic signaling molecule (Miller et al., 1994; Mithöfer et al., 1996). Recently it was proposed that defined short chain chitin oligomers from arbuscular mycorrhizal fungi function as a signal molecule in *M. truncatula* leading to nuclear Ca^{2+} spiking and activation of SYM-dependent signaling pathways (Genre et al., 2013). Additionally, like for the bacterial Nod-factors and the fungal Myc-factors, the signaling capability of β-glucans may among others depend on specific modifications, like sulfatations. Even after 40 years of β-glucan research in plant–microbe interactions there are still many open questions. Recent advancements in mammalian systems and the availability of new genomic information in plants could help to answer these questions.

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