Serine–arginine protein kinase-like protein, SrpkF, stimulates both cellobiose-responsive and d-xylose-responsive signaling pathways in *Aspergillus aculeatus*

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**Abstract**

*Aspergillus aculeatus* produces cellulolytic enzymes in the presence of their substrates. We screened a library of 12,000 *A. aculeatus* T-DNA-inserted mutants to identify a regulatory factor involved in the expression of their enzyme genes in response to inducers. We found one mutant that reduced the expression of FIII-avicelase (*chbI*) in response to cellulose. T-DNA was inserted into a putative protein kinase gene similar to AN10082 in *A. nidulans*, serine–arginine protein kinase F, SrpkF. Fold increases in *srpkF* gene expression in response to various carbon sources were 2.3 (d-xylose), 44 (Avicel®), 59 (Bacto™ Tryptone), and 98 (no carbon) compared with d-glucose. Deletion of *srpkF* in *A. aculeatus* resulted in a significant reduction in cellulose-responsive expression of *chbI*, hydrocellulase (*cel7b*), and FIb-xylanase (*xynIb*) genes at an early induction phase. Further, the *srpkF*-overexpressing strain showed upregulation of the *srpkF* gene from four- to nine-fold higher than in the control strain. *srpkF* overexpression upregulated *chbl* and *cel7b* in response to cellobiose and the FI-carboxymethyl cellulase gene (*cmc1*) and *xynIb* in response to d-xylose. However, the *srpkF* deletion did not affect the expression of *xynIb* in response to d-xylose due to the less expression of *srpkF* under the d-xylose condition. Our data demonstrate that SrpkF is primarily involved in cellulose-responsive expression, though it has a potential to stimulate gene expression in response to both cellobiose and d-xylose in *A. aculeatus*.

**Keywords** Gene regulation · Cellulase · ManR · XlnR · Filamentous fungi · Phosphorylation

**Introduction**

Lignocellulosic biomass has long been recognized as a potential sustainable source of mixed sugars for fermentation to biofuels and chemicals (Himmel et al. 2007). A key step for bioconversion of lignocellulose is enzymatic hydrolysis of pretreated lignocellulose to fermentable sugars. Filamentous fungi are prominent producers of enzymes that degrade lignocellulose (Payne et al. 2015). *Trichoderma reesei* is a well-known species that produces copious amounts of cellulolytic enzymes (Bischof et al. 2016). *T. reesei* glycoside hydrodases are being continuously improved to utilize lignocellulose as a feedstock for the generation of bio-based products. For example, *Aspergillus aculeatus* no. F-50 [NBRC 108789] was isolated from soil as a host for production of carbohydrate-active enzymes that cooperatively hydrolyze pulp in combination with *Trichoderma reesei* (Murao et al. 1979). β-Glucosidase from *A. aculeatus* no. F-50 was introduced into *T. reesei*, which accelerated cellulose hydrolysis (Baba et al. 2015; Nakazawa et al. 2012). This β-glucosidase showed high compatibility with *T. reesei*, suggesting that *A. aculeatus* produces promising enzymes for liberating fermentable sugars from lignocellulose. However, cellulolytic and xylanolytic enzymes of *A. aculeatus* are not utilized in industry because of low production levels. We aimed to understand the regulatory mechanisms of the associated genes and apply this knowledge to improving enzyme production in *A. aculeatus*.

Cellulolytic and xylanolytic enzyme production in *Aspergillus* is regulated at the transcriptional level. The first identified regulator of cellulolytic and xylanolytic genes was XlnR, a Zn(II)2Cys6-type transcriptional activator that
coordinates xylanolytic expression in *Aspergillus niger* (van Peij et al. 1998). Genetic analysis indicates that XlnR controls the expression of xylanolytic and cellulolytic enzyme genes in *A. aculeatus* (Kunitake and Kubayashi 2017; Tani et al. 2014). XlnR regulates expression of Flb-xylanase (*xynIb*) and FI-carboxymethyl cellulase (*cmc1*) genes in response to cellulose and d-xylene, respectively. In contrast, FIII-avicelase (*cbhI*), FII-carboxymethyl cellulase (*cmc2*), and hydrocellulase (*cel7b*) are induced in response to cellulose via an XlnR-independent signaling pathway in *A. aculeatus* (Tani et al. 2012). C1r-2 in *Neurospora crassa* and C1rB, a C1r-2 homolog in *Aspergillus nidulans*, were identified as Zn(II)2Cys6-type transcriptional activators that control expression of cellulolytic enzyme genes in response to cellulosic carbon sources (Coradetti et al. 2012). ManR, a C1rB ortholog, participates in the XlnR-independent signaling pathway in *Aspergillus oryzae* (Ogawa et al. 2013).

We further screened for a new regulator to better understand the regulatory mechanisms underlying ManR- and XlnR-dependent signaling pathways that modulate gene expression in response to cellulose. We identified a putative protein kinase, SrpkF, which increased expression of cellulolytic and xylanolytic genes in response to cellobiose and d-xylene under control of both ManR-dependent and XlnR-dependent signaling in *A. aculeatus*.

**Materials and methods**

**Strains, transformation, marker recycling, and T-DNA insertion**

All *A. aculeatus* strains used in this study were derived from wild-type *A. aculeatus* no. F-50 [NBRC 108789]. Unless otherwise stated, all strains were propagated at 30 °C in an appropriately supplemented MM (Adachi et al. 2009). *A. aculeatus* NCP2 (*niaD1::niaD::P cbhI/pyrG; pyrG1*) was used to construct *A. aculeatus* strains for T-DNA insertion via *Agrobacterium tumefaciens* mediated transformation. Counterselection on 5-FOA and marker recycling were performed as described previously (Kunitake et al. 2011, 2013). *A. aculeatus* MR12 (*pyrG1; Δku80*) was used for the disruption and complementation of the *srpkF* gene (Tani et al. 2013). *Escherichia coli* DH5αF was used for plasmid construction.

**Disruption and complementation of *srpkF***

The *A. aculeatus* *srpkF*-deficient mutant (*pyrG1; Δku80; Δ*srpkF*) was created by replacing *srpkF* with the *A. nidulans* orotidine 5′-phosphate decarboxylase gene (*AnpyrG*) followed by marker recycling (Tani et al. 2013). The *srpkF* deletion cassette was constructed with the 5′ and 3′ regions of *srpkF*, which are key in homologous recombination to replace *srpkF* with *AnpyrG* were amplified from *A. aculeatus* genomic DNA using primer pairs 2.6 k-5′*srpkF/F* 2.6 k-5′*srpkR* and L-kinaseF/L-kinaseR, respectively. The *AnpyrG* gene was amplified from *A. nidulans* genomic DNA using the primer pair AnpyrG-KF/AnpyrG-KR. The 3′ flanking region of *sepM* was amplified using the primer pair 2.6 k-MsrpkF/M-kinaseR to eliminate *AnpyrG* by intramolecular homologous recombination at the *srpkF* locus. The 5′ region of *AnpyrG* (responsible for marker recycling) and the 3′ region were fused via PCR using the primer pair 2.6 k-5′*srpkF/L-kinaseR* and subcloned
into the *Eco*RV site of pBluescriptIKS(+) to yield pDsrpkF. The *srpkF* deletion cassette was amplified via PCR using the primer pair 2.6 k-5'srpkF/L-kinaseR from pDsrpkF and introduced into MR12 (*pyrG1; Δku80*) using the protoplast-PEG method to yield the *A. aculeatus ΔsrpkF* plus *pyrG* strain (*pyrG1; Δku80; ΔsrpkF::AnpyrG*). Marker recycling used 1 × 10⁴ transformant spores spread onto MM supplemented with 0.01% uridine and 1 mM 5-FOA. *A. aculeatus ΔsrpkF* was transformed with amplified DNA fragments to yield the *srpkF*-overexpressing strain (Supplementary Fig. S3).

**Gene expression analysis by quantitative RT-PCR**

Quantitative RT-PCR (qRT-PCR) was used to quantify the expression of cellulase and hemicellulase genes as previously described (Tani et al. 2017). 0.1% Bacto™ Tryptone (Thermo Fisher Scientific, Tokyo, Japan) was used as a neutral carbon source (noninducing condition). Indicated carbon sources were added to media supplemented with 0.1% Bacto™ Tryptone to investigate the expression of test genes. Total RNA (500 ng) was used to amplify cDNA with RevertA-Tra Ace™ qPCR RT-Master Mix (Toyobo, Tokyo, Japan). qRT-PCR was performed in a Thermal Cycler Dice™ Real-Time System (Takara, Kyoto, Japan). For amplification, a SYBR® Green I assay using THUNDERBIRD™ SYBR® qPCR Mix (Toyobo) was performed in a 20 μl reaction. Primers used for qRT-PCR are listed in Supplementary Table S1. Expression of the glyceraldehyde-3-phosphate dehydrogenase A gene (*gpdA*) was used as an internal control. The specificity of the PCR amplification was confirmed by melting curve analysis. The expression profile of each gene was analyzed with the delta–deltaCT method. More than three biological replicates were performed for each experiment, and each replicate was evaluated in triplicate.

**Additional methods**

Genomic DNA preparation and Southern blotting were performed as described previously (Kunitake et al. 2013). An in-house *A. aculeatus* draft genome database was used to obtain the genomic sequence of the *srpkF* gene. Two independently amplified cDNA fragments were analyzed to determine the *srpkF* cDNA sequence. Conidia from the *A. aculeatus* strains were collected in a 0.1% Tween® 80/0.8% NaCl solution and counted using a hemocytometer. The number of conidia was normalized by colony area.

**Nucleotide sequence data**

Nucleotide sequence data were deposited in Japan’s DNA Data Bank (DDBJ) Nucleotide Sequence Data Libraries. The accession number of *srpkF* in *A. aculeatus* is DDBJ Acc. no. LC638744.
Results

Isolation of a cellulose-responsive induction-deficient mutant from an A. aculeatus T-DNA insertion mutant library

We previously screened an A. aculeatus T-DNA insertion mutant library of approximately 12,000 transformants for strains that were 5-FOA-resistant and cellulose-responsive induction-deficient. We isolated five 5-FOA-resistant strains that showed reduced growth on medium supplemented with 1% Avicel® but showed normal growth on media supplemented with 1% glucose, 1% beechwood xylan, or 1% d-xylose (Tani et al. 2017). We further analyzed one 5-FOA-resistant strain (Q3) that showed a strong correlation between the function of the gene disrupted by T-DNA and the cellulose-responsive induction-deficient phenotype.

We first investigated cbhI expression profiles in response to Avicel® in Q3 because the cbhI promoter was fused to the pyrG gene. This reporter gene was used as bait to screen for factors involved in the cellulose-induced signaling pathway with the 5-FOA-resistant phenotype (Kunitake et al. 2013). Expression of cbhI was induced in response to Avicel® at 9 h postinduction in the control strain (NCP2) but significantly reduced in Q3 to approximately 20% of NCP2 expression levels (p < 0.05, Student’s t test) (Fig. 1). Expression of xynIb in response to Avicel® was also significantly reduced in Q3 to approximately 30% of NCP2 expression levels (p < 0.05, Student’s t test) (Fig. 1). These data suggest that the T-DNA insertion in Q3 disrupted a gene required for the induction of test genes in response to Avicel®.

Serine–arginine protein kinase F participates in the early phase of cellulose induction

T-DNA integration into Q3 was analyzed by Southern blotting since the recovery of T-DNA flanking sequences by inverse PCR differs depending on integration pattern. Genomic DNA isolated from Q3 was digested with EcoRI, XbaI and SpeI, HindIII, and PstI. Single digestion by EcoRI (unique site in the T-DNA left flanking region), HindIII (absent from the vector), and PstI (unique site in the vector backbone) and double digestion with XbaI and SpeI (unique site in the T-DNA right flanking region and absent from the vector, respectively) all yielded a single band (Fig. 2a). These data demonstrate that one copy of T-DNA was integrated into the Q3 genome. We amplified the T-DNA flanking sequences via inverse PCR using Q3 genomic DNA digested with EcoRI and XbaI/SpeI to recover the right and left flanking sequences, respectively (Fig. 2b). We sequenced DNA fragments amplified via inverse PCR, which showed that the T-DNA integrated into the ORF encoding the putative serine–threonine protein kinase (Fig. 2b). The right and left flanking sequences of the T-DNA were inserted at 1122 and 1137 nt from the translation start site of the gene with a 15 bp deletion in the recipient genome. The putative protein

Fig. 1 Identification of a cellulose-responsive induction-deficient mutant. qRT-PCR analysis of cbhI and xynIb expression at 9 h postinduction with 1% (w/v) Avicel® was performed for control (NCP2) and Q3 strains. Relative expression corresponds to the ratio of the mean expression levels of cbhI divided by mean expression of gpdA, the reference gene. Relative expression levels are means of three independent experiments, and error bars indicate the standard deviations. Letters indicate significant differences between groups (p < 0.05, Student’s t test)

Fig. 2 Determination of the T-DNA integration pattern and identification of the disrupted gene. Deduced T-DNA integration via Southern blotting (a) and a schematic representation of the SrpkF locus (b)
kinase gene was composed of 1443 bp with five exons and encoded a 416-amino acid protein. Based on a homology search in FungiDB (https://fungidb.org/fungidb/), the putative protein kinase was most similar to ACLA_003920 in Aspergillus clavatus (E value = 0.0; Identities = 81%). The gene was also similar to AN10082 in A. nidulans. SrpkF (E value = 7e⁻¹⁰⁰; Identities = 38%). This protein is a member of a family of serine–threonine protein kinases that includes an expanded group of seven serine–arginine protein kinases (SRPK) in A. nidulans (de Souza et al. 2013). Therefore, this gene was designated srpkF in A. aculeatus. The T-DNA insertion at 1122 nt from the translation start site caused production of a truncated protein of 327 amino acids in the original protein and seven additional amino acids (SNTD-). The frame shift at 1122 nt from the translation start site caused a truncation at 1129 nt leading to a stop codon derived from the T-DNA fragment. Thus, srpkF is the candidate gene causing the cellulose-responsive induction-deficient phenotype of Q3.

The srpkF gene expressed under the carbon starvation condition

Expression profiles of srpkF were assessed under inducing (1% Avicel® or 1% α-xylene), repressing (1% ρ-glucose), carbon neutral (0.1% Bacto™ Tryptone), and carbon starvation conditions of the cellulolytic and xylanolytic enzyme expression in wild-type A. aculeatus. Transcripts of srpkF were quantified using RNA prepared from A. aculeatus wild-type strain grown under the following conditions: 1% ρ-glucose medium for 3 h, 1% α-xylene medium for 3 h, 1% Avicel® medium for 9 h, 0.1% Bacto™ Tryptone medium for 3 h, and no carbon source for 3 h. Fold increases in srpkF gene expression compared with ρ-glucose medium were 2.3 (α-xylene), 44 (Avicel®), 59 (Bacto™ Tryptone), and 98 (no carbon) (Fig. 3). A. aculeatus grew poorly in 1% ρ-glucose medium, 0.1% Bacto™ Tryptone medium, and under the no carbon condition, whereas it grew well on 1% glucose and 1% α-xylene for 3 h, indicating that A. aculeatus did not require sufficient carbon from 1% Avicel® and 0.1% Bacto™ Tryptone media. These data indicate that srpkF is expressed under conditions in which available carbon is limited.

Functional analysis of SrpkF in A. aculeatus

To genetically analyze the function of srpkF, the entire srpkF gene was deleted by homologous recombination (ΔsrpkF, pyrGΔ) followed by marker recycling to yield an A. aculeatus srpkF deletion mutant (ΔsrpkF, pyrGΔ). A srpkF deletion mutant was never isolated using the DNA fragment corresponding to the 1522 nt region upstream from the translation start site that was used for homologous recombination. Thus, the DNA fragment from 1523 to 2613 nt upstream was targeted. Further, the 3′ flanking region of srpkF was deleted after marker recycling in ΔsrpkF. Thus, the srpkF deletion mutant included elimination of 1.5 kb upstream and 1.3 kb downstream flanking sequences. ΔsrpkF was transformed with srpkF DNA fragments yielding a complemented strain (srpkF+) (Supplementary Fig. S1). A C-terminal deletion mutant of SrpkF composed of 327 amino acids (ΔCsrpkF) in Q3 was generated by introducing a stop codon at 1129 nt from the translation start site of the ORF, followed by marker recycling of pyrG to yield ΔCsrpkF (SrpkF1-327, pyrGΔ). ΔCsrpkF was also complemented using the srpkF DNA fragment. We confirmed that gene replacement and complementation occurred as expected by Southern blotting (Supplementary Fig. S2). MR12, ΔsrpkF, ΔCsrpkF, and srpkF+ grew equally well on MM supplemented with 1% ρ-glucose, 1% α-xylene, and 1% Avicel® (data not shown). Expression profiles of test genes in the complemented ΔCsrpkF strain were not different from profiles from MR12 and srpkF+, and we thus used srpkF+ as a control strain (data not shown).

We investigated the effect of srpkF deletion on gene expression in response to cellulosic carbon sources. Transcripts of cbhl and xynlb were quantified at 6 and 9 h postinduction in MR12, ΔCsrpkF, ΔsrpkF, and srpkF+ strains. Expression of cbhl and xynlb was reduced in ΔCsrpkF and ΔsrpkF only after induction. Fold induction of cbhl in response to Avicel® decreased significantly to 50% in ΔsrpkF and 31% in ΔCsrpkF of the response in MR12 at 9 h postinduction (p < 0.05, Student’s t test). Similarly, fold induction of xynlb significantly decreased to 26% in ΔsrpkF and 32% in ΔCsrpkF (p < 0.05, Student’s t test) (Supplementary Fig. S4 and Fig. 4). SrpkF participates in the expression of both genes in response to Avicel®. Expression levels decreased similarly in both ΔCsrpkF and ΔsrpkF and were restored by srpkF complementation. Hence, we further
analyzed the SrpkF function using only ΔsrpkF. Expression of cel7b in ΔsrpkF decreased significantly to 55% of expression in MR12 at 9 h postinduction (p < 0.05, Student’s t test), which was restored in srpkF+ (Fig. 4). The expression of cmc2, cmc1, and xynIa, decreased to approximately 60%, but these reductions were not statistically significant (Fig. 4).

SrpkF participates in cellulose-responsive expression of cellulosytic and xylanolytic enzyme genes in A. aculeatus

To assess the effect of overexpression of srpkF on the expression of genes encoding cellulosic biomass-degrading enzymes in A. aculeatus, we constructed an srpkF-overexpressing strain that constitutively expresses srpkF under the control of the translation elongation factor 1α gene promoter (Ptef), a high-level constitutive promoter in Aspergillus (Kunitake et al. 2015). The srpkF-overexpressing cassette was introduced into the pyrG locus in ΔsrpkF as a single copy by homologous recombination, as confirmed by Southern blotting (Supplementary Fig. S3), yielding the srpkF-overexpressing strain (OEsrpkF). Expression of srpkF increased four to seven-fold in the presence of cellulbiose with 1-deoxynojirimycin (DNJ) and approximately nine-fold in the presence of D-xylene (Supplementary Fig. S5). Since A. aculeatus produces β-glucosidase, which effectively hydrolyzes cellulbiose to glucose, DNJ was added as a β-glucosidase inhibitor (Tani et al. 2012). The physiological phenotype of the OEsrpkF strain was no different from MR12, ΔCsrpkF, and ΔsrpkF (data not shown).

We assessed the effect of srpkF overexpression on the expression of cellulosic biomass-degrading enzyme genes in response to physiological inducers. cbhI, cmc2, and cel7b were upregulated in response to cellulbiose via ManR-dependent signaling. Expression of cbhI, cmc2, and cel7b generally increased in OEsrpkF compared with MR12 under inducing conditions (Fig. 5a, b); however, expression profiles varied. Expression of cbhI was induced at 2, 3, and 4 h postinduction in OEsrpkF and resulted in a significant fold increase (Fig. 5a, b). By contrast, overexpression of srpkF did not significantly increase either expression or fold induction of cmc2 (Fig. 5a, b). Expression of cel7b was significantly stimulated under both inducing and noninducing conditions and showed no significant difference in the fold induction. Thus, SprkF stimulated expression of cel7b at a basal level (Fig. 5a, b).

Expression of xynIb and cmc1 is induced in response to D-xylene via XlnR-dependent signaling. Expression of xynIb and cmc1 was enhanced markedly at 1.5 and 3.0 h postinduction with D-xylene in OEsrpkF (Fig. 5c, d). These data
confirm that SrpkF has a potential to participate in cellulose- and d-xylose-responsive signaling pathways.

**Discussion**

We identified SrpkF as a positive regulator that induces cellulolytic and xylanolytic enzyme gene expression in response to cellulose. An *srpkF*-overexpressing strain demonstrated the potential for this gene to stimulate the d-xylose-responsive induction via the XlnR-dependent signaling pathway. However, SrpkF functions as a positive regulator to stimulate cellulose-responsive induction via both ManR- and XlnR-dependent signaling under physiological conditions. *srpkF* expression was stimulated under cellulose and carbon starvation condition but not by the presence of d-xylose (Fig. 6).

A comparison of amino acid sequences using the FASTA algorithm indicated that *srpkF* orthologs are highly conserved in the *Aspergillus* section *Nigri*. Orthologs are also present in some strains from other sections and other genera, such as *A. nidulans* (AN10082, *E* value = 2e-91; Identities = 42%), *Penicillium rubens* (Pc12g16110, *E* value = 1e-104; Identities = 47%), and *Coccidioides immitis* (CIMG_04484, *E* value = 6e-103; Identities = 44%) (Fig. 7). AN10082 in *A. nidulans* encodes a serine–arginine protein kinase F (SrpkF) for which a deletion mutant did not show a distinguishing phenotype (de Souza et al. 2013). SRPK was first identified as a cell cycle-regulated kinase specific for SR proteins, which are a family of pre-mRNA splicing factors.

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**Fig. 5** Effect of *srpkF* overexpression on expression of cellulase and hemicellulase genes. qRT-PCR results for each gene in MR12 (M, black bars) and the *srpkF*-overexpressing strain OEsrpkF (O, striped bars). a, b RNA was prepared from strains grown for 2–4 h in the presence of 0.1% cellobiose with 50 μg/L DNJ. Relative expression corresponds to the ratio of each gene divided by mean expression levels of *gpdA* (a). Fold induction of each test gene reflects the gene expression level under inducing conditions divided by expression under noninducing condition (b). c, d RNA was prepared from strains grown for 1.5–3.0 h in the presence of 1% (w/v) D-xylose. Relative expression corresponds to the ratio of each gene divided by mean expression levels of *gpdA* (c). d Fold induction of each test gene reflects gene expression under inducing conditions divided by expression under noninducing conditions (d). For all panels, the results shown are the means of three independent experiments, and the error bars indicate the standard deviations. An asterisk indicates a significant difference between the expression of test genes in MR12 and OEsrpkF (*p* < 0.05, Student’s *t* test).
containing SR domains that consist largely of serine/arginine repeats (Gui et al. 1994a, b). Members of this kinase family are known to phosphorylate serines within SR domains and are widely conserved in eukaryotes. *S. cerevisiae* encodes a single SRPK family member, Sky1, but *A. nidulans* and *A. aculeatus* encode seven SRPKs from SrpkA to G (de Souza et al. 2013) and four SRPKs, respectively. SrpkA proteins in *Aspergillus* are highly conserved along with *S. cerevisiae* Sky1. Thus, these proteins may modulate subcellular localization and function of Ser-Arg rich splicing-factor proteins (Dagher and Fu 2001; Gui et al. 1994a). However, SRPKs possess various domains, suggesting functional diversification. Few non-splicing functions of SRPKs are reported (Gou et al. 2020; Hong et al. 2012; Wang et al. 2017), and it remains unclear whether SRPKs have evolved further regulatory roles (Bustos et al. 2020). Functions of SRPKs might be illuminated by close examination of gene expression since some genes exhibited highly tissue-specific profiles (Nakagawa et al. 2005; Wang et al. 1998).

The expression of srpkF increased under carbon-limited conditions, such as the no carbon and Avicel® conditions (Fig. 3), which showed strong correlations between SrpkF functions in cellulose-responsive induction and expression profiles. We focused on two signaling pathways involved in cellulose-responsive induction in *A. aculeatus* to narrow possible pathways. One is ManR-dependent signaling that induces expression of *cbhI*, *cmc2*, and *cel7b*. We expected that deletion and overexpression of srpkF would show a consistent effect on these three genes. However, effects varied, suggesting that transcription factors other than ManR could be involved. Overexpression of *clbR*, a putative transcription factor involved in cellulose-responsive induction in *A. aculeatus*, did not affect the expression of *cbhI* and *cmc2* but did reduce the expression of *cel7b*. Still, deletion of *clbR* reduced their expression levels in response to Avicel® (Kunitake et al. 2015). These data suggest that various factors participate in ManR-dependent signaling in response to cellulasic carbon sources in *A. aculeatus*.

Interestingly, overexpression of srpkF promoted xylanase gene expression in response to d-xylose, but no effect of srpkF deletion was seen for xylose-responsive expression of the xylanase gene. SrpkF is thus critically regulated at the transcription level. How SrpkF participates in the two different signaling pathways in response to cellulose and d-xylose is not clear. XlnR is constitutively expressed in *A. oryzae* and is phosphorylated in the presence of d-xylose then rapidly dephosphorylated by removing d-xylose from the medium (Noguchi et al. 2011). ManR phosphorylation status is unknown and SrpkF is a candidate kinase that could be involved in ManR- and XlnR-dependent signaling (Fig. 6).

A recent study addressed levels of conservation and diversity in the regulatory mechanisms of cellulolytic enzyme genes in Ascomycete fungi (Kunitake and Kobayashi 2017). XlnR controls the transcription of 20–30 genes encoding...
cellulolytic and xylanolytic enzymes in the presence of cellulose in *A. niger* (Stricker et al. 2008). XlnR mainly regulates the expression of xylanolytic enzyme genes for *A. oryzae* and *A. aculeatus* and is only marginally involved in the expression of cellulolytic enzyme genes in response to cellulose (Marui et al. 2002; Tani et al. 2012). In contrast, Xyr1, an XlnR ortholog in *T. reesei*, is a master regulator that modulates the expression of xylanolytic and cellulolytic enzyme genes in response to various carbon sources, such as d-xylose, sorphorose, galactose, and lactose (Stricker et al. 2006, 2007). XLR-1 in *N. crassa* participates in the induction of xylanolytic but not significantly involved in the induction of cellulolytic enzyme genes (Sun et al. 2012).

The expression of cellulolytic enzyme genes is mainly regulated by ManR in *A. oryzae* and *A. aculeatus* (Ogawa et al. 2013; Tsumura et al. 2021) and its orthologs ClrB in *N. nidulans* and CLR-2 in *N. crassa* (Coradetti et al. 2012, 2013). Complex regulation mechanisms can be conferred by the acquisition of paralogous genes that establish new signal transduction pathways (Baker et al. 2013). Orthologous genes of *srpkF* are absent from the genomes of several cellulase-producing fungi, such as *Trichoderma* and *Neurospora* species. The acquisition of *srpkF* in *Aspergillus* might lead to differential regulation of cellulolytic enzyme genes. A logical next step is to identify target proteins of SrpkF, which will help understand the complex regulatory mechanisms of cellulolytic enzyme genes in *Aspergillus*.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00294-021-01207-x.

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**Declarations**

**Conflict of interest** The authors have no conflicts of interest to declare that are relevant to the content of this article.

**Ethical approval** This article does not contain any studies that involve human participants or animals.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Data availability** All data generated or analyzed during this study are included in this published article and its Supplementary Information files. DNA sequences will be available at the DDBJ database when this article is released for publication.

**Code availability** Not applicable.

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