Phytosulfokine peptide signalling

Margret Sauter*

Plant Developmental Biology and Plant Physiology, University of Kiel, Am Botanischen Garten 5, 24118 Kiel, Germany

* To whom correspondence should be addressed. E-mail: msauter@bot.uni-kiel.de

Received 2 December 2014; Revised 9 January 2015; Accepted 27 January 2015

Abstract

Phytosulfokine (PSK) belongs to the group of plant peptide growth factors. It is a disulfated pentapeptide encoded by precursor genes that are ubiquitously present in higher plants, suggestive of universal functions. Processing of the preproprotein involves sulfonylation by a tyrosylprotein sulfotransferase in the trans-golgi and proteolytic cleavage in the apoplast. The secreted peptide is perceived at the cell surface by a membrane-bound receptor kinase of the leucine-rich repeat family. The PSK receptor PSKR1 from Arabidopsis thaliana is an active kinase and has guanylate cyclase activity resulting in dual-signal outputs. Receptor activity is regulated by calmodulin. While PSK may be an autocrine growth factor, it also acts non-cell autonomously by promoting growth of cells that are receptor-deficient. In planta, PSK has multiple functions. It promotes cell growth, acts in the quiescent centre cells of the root apical meristem, contributes to funicular pollen tube guidance, and differentially alters immune responses depending on the pathogen. It has been suggested that PSK integrates growth and defence signals to balance the competing metabolic costs of these responses. This review summarizes our current understanding of PSK synthesis, signalling, and activity.

Key words: Calmodulin, cell elongation, cGMP, defence, guanylate cyclase, leucine-rich repeat receptor kinase, phosphorylation, phytosulfokine, reproduction, sulfated peptide growth factor.

Introduction

Growth and development of plants is strongly influenced by environmental conditions. This plasticity allows plants to adapt to a changing and often adverse environment, thus compensating for their immobile lifestyle. Responses to environmental or developmental conditions are in many cases mediated by non-proteinaceous phytohormones. It has, however, become very clear that peptides are similarly important in plant signalling (Boller, 2005; Stahl and Simon, 2012; Czyzewicz et al., 2013; Matsubayashi, 2014). Phytosulfokine (PSK) belongs to a group of sulfated plant peptides that are bioactive at nanomolar concentrations. In Arabidopsis thaliana the disulfated pentapeptide PSK (Matsubayashi and Sakagami, 1996) and the 18-amino acid plant peptide containing sulfated tyrosine 1 (PSY1), which has a sulfated Tyr (Y2SO_3H) residue and a tri-L-arabinose side chain attached to a hydroxyproline residue (hP16tri-L-ara) (Amano et al., 2007), have been classified as growth factors; however, their functions, as we now know, far exceed growth regulation and include biotic and abiotic stress responses, and reproductive processes. This review focuses on the synthesis, perception, and physiological functions of PSK.

PSK synthesis

PSK was identified as a result of a stringent phenotype analysis and a biochemical purification procedure that was well thought through and executed (Matsubayashi and Sakagami, 1996). Plant cells kept in suspension require a minimum density to undergo cell division. This population effect can be overcome by the addition of medium from an older cell culture: this priming is known as feeder effect.
Matsubayashi and Sakagami (1996) established a robust bioassay to measure the activity of the chemical compound responsible for this feeder effect in suspension-cultured mesophyll cells from *Asparagus officinalis*, which led to the identification of two peptides via Edman degradation and mass spectrometry. The two activities that were purified from asparagus were identified as the disulfated pentapeptide Tyr(SO$_3$H)-Ile-Tyr(SO$_3$H)-Thr-Gln, termed PSK-$\alpha$, and its C-terminally truncated disulfated tetrapeptide PSK-$\beta$ (Tyr(SO$_3$H)-Ile-Tyr(SO$_3$H)-Thr). PSK-$\beta$ was eventually classified as a degradation product (Yang et al., 1999) and PSK was subsequently used as an equivalent term for PSK-$\alpha$. PSK-$\alpha$ promotes half-maximal growth of low-density suspension cells at 3.8 nM, whereas PSK-$\beta$ has a half-maximal activity at 37 nM (Matsubayashi and Sakagami, 1996). In wild-type *Arabidopsis* seedlings primary root growth is significantly promoted at 1 nM PSK (Stührwohldt et al., 2011). The tyrosylprotein sulfotransferase (TPST) that sulfates the PSK precursor is encoded by a single gene in *Arabidopsis* (Komori et al., 2009). In the *tpst-1* background with no endogenous active PSK root elongation is significantly promoted at 0.3 nM and higher concentrations of PSK, indicating high-affinity binding of PSK to its receptor. Analysis of 12 PSK analogues confirmed specificity of the disulfated pentapeptide and revealed a core requirement for the disulfated Tyr(SO$_3$H)-Ile-Tyr(SO$_3$H) N-terminal tripeptide (Matsubayashi et al., 1996). The unsulfated pentapeptide is not active below 100 nM in promoting division of cells at low density (Matsubayashi and Sakagami, 1996) and shows weak root growth promotion at 1 μM (Kutschmar et al., 2009). Hence, sulfonation of the two tyrosyl side chains of PSK increases its biological activity by about 1000-fold.

PSK is synthesized from 80–120 amino acid prepropeptides that are encoded by small gene families (Table 1) (Yang et al., 2001; Lorbiecke and Sauter, 2002). The cDNA of rice (*Oryza sativa*) OsPSK was mutated to encode the modified pentapeptide YIY$\alpha$Q, which was subsequently isolated from the culture medium of transformed cells providing proof that the transcript is processed to the mature peptide. The C-terminally truncated tetrapeptide YIY$\alpha$S was also present in the culture medium indicating that PSK-$\beta$ is a degradation product of PSK-$\alpha$ (Yang et al., 1999). A signal peptide targets the propeptide to the secretory pathway. The propeptide is little conserved at the primary sequence level, with the exception of a signature motif in the C-terminal half including the YIYTQ peptide (Fig. 1) (Lorbiecke and Sauter, 2002). This signature motif overlaps with an acidic region in the middle part of the propeptide. A conserved aspartate residue at the −1 position of the YIYTQ sequence was shown to be essential for sulfonation of rice OsPSK (Hanai et al., 2000). Unlike the primary amino acid sequence, the predicted secondary structures of PSK propeptides are well conserved. The postleader sequence is folded into three $\alpha$-helices including a conserved helix1-turn-helix2 motif (Lorbiecke and Sauter, 2002). A requirement of these structural features for propeptide processing has been proposed but awaits verification.

The TPST responsible for sulfonation is a membrane-bound enzyme localized in the *trans*-Golgi network, probably as a glycosylated protein (Komori et al., 2009). TPST was identified in *Arabidopsis* using a peptide modelled according to the PSY1 (‘plant peptide containing sulfated tyrosine 1’) propeptide. The PSY1 peptide is 18 amino acids in length, derived from a larger precursor, and sulfated as well as glycosylated (Amano et al., 2007). TPST was subsequently shown

### Table 1. Details of PSK precursor genes, PSK receptor genes, and the tyrosylprotein sulfotransferase gene from *Arabidopsis*, and predicted homologues from rice

<table>
<thead>
<tr>
<th>Gene locus</th>
<th>Encoded protein (amino acids)</th>
<th>Name</th>
<th>Function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Os06g0633300</td>
<td>89</td>
<td>OsPSK1 (OsPSK)</td>
<td>Preproprotein</td>
<td>1, 2</td>
</tr>
<tr>
<td>Os11g0149400</td>
<td>119</td>
<td>OsPSK2</td>
<td>Preproprotein</td>
<td>2</td>
</tr>
<tr>
<td>Os12g0147800</td>
<td>102</td>
<td>OsPSK3</td>
<td>Preproprotein</td>
<td>2</td>
</tr>
<tr>
<td>Os07g0124100</td>
<td>83</td>
<td>OsPSK4</td>
<td>Preproprotein</td>
<td>2</td>
</tr>
<tr>
<td>Os03g0675600</td>
<td>75</td>
<td>OsPSK5</td>
<td>Preproprotein</td>
<td>2</td>
</tr>
<tr>
<td>Os03g0232400</td>
<td>101</td>
<td>OsPSK6</td>
<td>Preproprotein</td>
<td>3</td>
</tr>
<tr>
<td>Os11g0557000</td>
<td>94</td>
<td>OsPSK7</td>
<td>Preproprotein</td>
<td>3</td>
</tr>
<tr>
<td>LOC_Os02g41890.1$^a$</td>
<td>1052</td>
<td>OsPSKR1</td>
<td>LRR-RLK receptor</td>
<td>3</td>
</tr>
<tr>
<td>LOC_Os04g57630.1$^a$</td>
<td>1035</td>
<td>OsPSKR2</td>
<td>LRR-RLK receptor</td>
<td>3</td>
</tr>
<tr>
<td>At1g13590</td>
<td>87</td>
<td>AIPSK1</td>
<td>Preproprotein</td>
<td>1, 2</td>
</tr>
<tr>
<td>At2g22860</td>
<td>87</td>
<td>AIPSK2</td>
<td>Preproprotein</td>
<td>1, 2</td>
</tr>
<tr>
<td>At3g44735</td>
<td>81</td>
<td>AIPSK3</td>
<td>Preproprotein</td>
<td>1, 4</td>
</tr>
<tr>
<td>At3g49780</td>
<td>87</td>
<td>AIPSK4</td>
<td>Preproprotein</td>
<td>5</td>
</tr>
<tr>
<td>At5g65870</td>
<td>77</td>
<td>AIPSK5</td>
<td>Preproprotein</td>
<td>2</td>
</tr>
<tr>
<td>At4g37720</td>
<td>87</td>
<td>AIPSK6</td>
<td>Preproprotein</td>
<td>6</td>
</tr>
<tr>
<td>At2g02220</td>
<td>1008</td>
<td>AIPSKR1</td>
<td>LRR-RLK receptor</td>
<td>7</td>
</tr>
<tr>
<td>At5g53890</td>
<td>1036</td>
<td>AIPSKR2</td>
<td>LRR-RLK receptor</td>
<td>7</td>
</tr>
<tr>
<td>At1g08030</td>
<td>500</td>
<td>AITPST</td>
<td>Sulfotransferase</td>
<td>8</td>
</tr>
</tbody>
</table>

$^a$ Based on Phytozome data.

References: (1) Yang et al., 1999; (2) Lorbiecke and Sauter, 2002; (3) this review; (4) PSK6 in Lorbiecke and Sauter, 2002; (5) PSK3 in Lorbiecke and Sauter, 2002; (6) PSK4 in Lorbiecke and Sauter, 2002; (7) Matsubayashi et al., 2002; (8) Komori et al., 2009.
also to accept a PSK precursor as a substrate and to catalyse sulfonylation of root growth factors (RGFs) (Amano et al., 2007; Komori et al., 2009; Matsuzaki et al., 2010). RGFs were independently identified and described as CLV3/ESR-related-like (CLEL) peptides and were suggested to be active as unsulfated peptides (Meng et al., 2012). TPST activities in microsomal fractions from asparagus, rice, carrot (Daucus carota), tomato (Solanum lycopersicum), and tobacco (Nicotiana tabacum) vary greatly when peptides of various lengths modified after the OsPSK N-terminal sequence are used as substrates, with the sulfate group donor 3'-phospho-5'-adenylsulfate (PAPS) as a co-substrate (Hanaei et al., 2000). The transporter responsible for translocating PAPS into the Golgi has not been identified so far. In Arabidopsis, TPST is encoded by a single gene. The loss-of function tsp1-1 mutant shows pleiotropic and severe developmental defects (Komori et al., 2009; Zhou et al., 2010). In roots, maintenance of the quiescent centre (QC), meristem, and columnella stem cell identity, and co-ordinated columnella stem cell division, is dependent on TPST-mediated activation of RGFs. The transcription factor genes PLETHORAI and PLETHORA2 mediate these TPST/RGB responses (Matsuzaki et al., 2010; Zhou et al., 2010).

Currently available data suggest that the sulfated PSK propeptide is proteolytically processed in the apoplast. The subtilase AtSBT1.1 of Arabidopsis localizes to the apoplast (Srivastava et al., 2008). It cleaves the AtPSK4 propeptide at the –3 position of the PSK pentapeptide sequence. Exopeptidases may further process the AtSBT1.1 product but these have not been identified. AtSBT1.1 displays specificity for the AtPSK4 propeptide and processes other propeptides less efficiently (PSK2, PSK5) or not at all (AtPSK1, AtPSK3, AtPSK6), suggesting that more of the 56 members of the subtilase gene family (Rautengarten et al., 2005) are engaged in PSK propeptide processing. Proteolytic processing of PSK propeptides may limit PSK synthesis. Transgenic Arabidopsis seedlings overexpressing C-terminally 4mmyc-tagged AtPSK4 propeptide did not display proteolytic activity. AtSBT1.1 transcripts and AtPSK4 propeptide cleavage were, however, induced within 5 and 8h, respectively, after excision of roots and transfer to conditioning medium (Srivastava et al., 2008), suggesting that synthesis of active PSK was induced through transcriptional activation of AtSBT1.1. Inactivating enzymes or transport mechanisms have so far not been reported for sulfated peptides.

**Regulation of PSK precursor and PSK receptor gene expression**

PSK genes are encoded by small gene families that are present in dicots and monocot angiosperms (Table 1) (Yang et al., 2001; Lorbiecke and Sauter, 2002). In Arabidopsis, five expressed PSK genes exist that contain the canonical YIYTQ sequence. A sixth gene, AtPSK6 (At4g37720); it is identical to AtPSK4 in Lorbiecke and Sauter, 2002) encodes the pentapeptide YIYTH. No ESTs or cDNAs have been reported for it, indicating that it is not expressed. PSK precursor genes have not been reported for lower plants (Lorbiecke and Sauter, 2002), suggesting that PSK signalling may have co-evolved with tissue diversification and the development of complex organs.

PSK precursor genes display differential expression throughout the plant life cycle. The cell and tissue type-specific expression of each gene points to a defined role of PSK signalling in defined tissues and suggests that PSK acts locally. Of the five Arabidopsis genes, AtPSK1 is expressed in a root-specific manner, whereas all the other genes are expressed in the root and shoot (Matsubayashi et al., 2006; Kutschmar et al., 2009). Expression in growing tissues agrees with the described function of PSK in promoting growth (see below). By contrast, the expression of AtPSK2, AtPSK4, and AtPSK5 in the central cylinder and of AtPSK1 in the root cap are not yet understood (Kutschmar et al., 2009), and may point to as yet unrecognized functions of PSK signalling. Specific and differential expression of PSK precursor genes also occurs in the Arabidopsis flower (Stührwohldt et al., 2015) and in the anthers and embryo sac of maize (Zea mays; Lorbiecke et al., 2005), supporting a role for PSK signalling in sexual reproduction of monocot and dicot plants. Interestingly, expression of AtPSK2 is induced in the ovule following fertilization, indicative of PSK signalling during early embryogenesis (Stührwohldt et al., 2015). Expression of PSK receptor genes is more difficult to assess as they are expressed at low levels. Arabidopsis has two PSK receptor genes, AtPSKR1 and AtPSKR2. AtPSKR1 appears to be expressed in all organs with varying abundance (Matsubayashi et al., 2006; Kutschmar et al., 2009; Stührwohldt et al., 2015) and is induced upon infection or elicitor treatment, as are AtPSK2 and AtPSK5 (Loihamäki et al., 2010; Igarashi et al., 2012). AtPSK3, AtPSK4, AtPSK5, and AtPSKR1 are also upregulated upon wounding (Matsubayashi et al., 2006; Loihamäki et al., 2010). Expression of AtPSKR2 has so far only been reported for flowers, where it is active in the carpel (Stührwohldt et al., 2015). Taken together, PSK precursor and receptor genes are subject to transcriptional regulation
by developmental and environmental signals, supporting a role of PSK signalling in growth and development, and in adaptation to biotic and abiotic stress.

**PSK is perceived by leucine-rich repeat receptor-like kinases at the plasma membrane**

PSK receptors are transmembrane proteins with an extracellular domain and a C-terminal intracellular kinase domain (Fig. 2). They belong to the family of receptor-like kinases (RLKs), with more than 600 members in *Arabidopsis* and over 1100 members in rice (Shiu and Bleecker, 2002; Morillo and Tax, 2006). The PSK receptor proteins are targeted to the secretory pathway by an N-terminal signal peptide, and anchored to the plasma membrane by a single transmembrane helix (Matsubayashi et al., 2002). Binding of PSK to a membrane fraction enriched in plasma membranes of rice suspension cells and to plasma membrane preparations of maize, asparagus, carrot, tobacco, and tomato was demonstrated using photoaffinity labelling and radiolabelled PSK (Matsubayashi et al., 1997; Matsubayashi and Sakagami 1999, 2000). Localization to the plasma membrane was later confirmed by fluorescence microscopy in *Arabidopsis* protoplasts expressing PSKR1-GFP or PSKR2-GFP fusion proteins (Hartmann et al., 2014).

The extracellular domain of PSK receptors consists of a leucine-rich repeat (LRR) region with 21 LRRs (Matsubayashi et al., 2002). PSK receptors are classified as members of the LRR-RLK X subclass (Morillo and Tax, 2006). The extracellular region is N-glycosylated, increasing the molecular mass by about 10 kDa (Matsubayashi and Sakagami 2000; Matsubayashi et al., 2002). LRRs 17 and 18 are intercepted by a 36-amino acid island domain (Matsubayashi et al., 2002). Photoaffinity labelling of the ectopically expressed extracellular region of the carrot PSK receptor DcPSKR1 with an iodine-labelled PSK analogue followed by immunoprecipitation of a tryptic digest and mass spectrometry led to the identification of a 15-amino acid fragment of the island domain (Glu503-Lys517) as a ligand binding site (Shinohara et al., 2007). Deletion analysis of this and an adjacent fragment in DcPSKR1 revealed that the entire island domain contributes to PSK binding. The charged ligand and sensitivity of ligand binding to ionic strength suggest that ligand binding occurs through ionic interactions (Matsubayashi and Sakagami, 1999). The island domain of the brassinosteroid receptor BRASSINOSTEROID INSENSITIVE 1 (BR1), with which the PSK receptor shares sequence and structural homology, is likewise involved in ligand binding. Mutations in the island domain of BRI1 result in reduced brassinolide binding (Torii, 2004).

Two binding sites have been described for PSK (Table 2). Rice membrane vesicles have a high-affinity binding site with a $K_D$ of 1.4 nM and a low-affinity binding site with a $K_D$ of 27 nM. Suspension-cultured rice cells bind PSK with $K_D$ values of 1 and 100 nM, respectively, with a 10-fold higher abundance of the low-affinity binding site (Matsubayashi and Sakagami, 1999). In carrot, high-affinity binding was attributed to two glycosylated proteins of 120 and 160 kDa that share the common PSK receptor protein backbone (Matsubayashi and Sakagami, 2000; Matsubayashi et al., 2002). The receptor protein has a molecular mass of 109.5 kDa, excluding the signal sequence. N-linked sugars that are cleaved by peptide N-glycosidase F (PNGase F) account for 10 kDa, resulting in a total mass of 120 kDa. The 160 kDa protein consists of the 109.5 kDa protein and a 10 kDa sugar modification. The molecular modification that accounts for the ~40 kDa difference in mass is unknown. Two receptor isoforms of 120 and 150 kDa molecular mass were also reported for *Arabidopsis* AtPSKR1 (Matsubayashi et al., 2006). The presence of PSK binding sites in monocot and dicot plants agrees with the widespread presence of PSK precursor genes and PSK receptor gene homologues in higher plants, and supports the idea that PSK signalling is ubiquitous in higher plants.

The concentration of PSK in the medium of suspension-cultured rice cells was reported at 34 nM (Matsubayashi et al., 1997). Mitogenic activity of asparagus mesophyll cells is induced to 50% at 3.8 nM PSK (Matsubayashi and Sakagami, 1996). In *Arabidopsis*, a half-maximal promotion of root elongation occurs at 1 nM PSK and of hypocotyl elongation at 3 nM PSK (Stührwohldt et al., 2011). These physiological responses in the nanomolar range agree well with high-affinity PSK binding. The PSK receptor null mutant of *Arabidopsis* is unresponsive to PSK (Igarashi et al., 2012), suggesting that both high- and low-affinity PSK binding are intrinsic to PSK receptors, possibly due to different configurations of the island domain or an additional as yet unidentified binding

![Fig. 2. PSK is perceived at the cell surface by a receptor kinase. The receptor protein possesses an extracellular glycosylated (light green shading) LRR domain with 21 LRRs (green bars) and an island domain in LRR 18 (pink circle) to which the PSK peptide binds. A transmembrane region flanked by juxtamembrane domains on either side links the perception domain to an intracellular Ser/Thr kinase (orange ellipse) that, in addition, has an intrinsic GC activity that produces cGMP. Regulation of the kinase occurs through calmodulin (CaM) binding. Signalling of growth and defence predictably involves gene regulation, but may also be more direct through regulation of soluble or membrane-localized targets of cGMP and/or kinase activity.](image-url)
site in the extracellular domain. Alternatively, PSK binds to another receptor with low affinity and this binding triggers different responses. Binding of a ligand to different receptors, as well as binding of different ligands to one receptor, would allow for signal crosstalk as well as signal integration.

PSK binding to rice plasma membranes is saturable, reversible, and specific for the disulfated pentapeptide. Unsulfated PSK competes weakly with PSK (Matsubayashi and Sakagami, 1999), in agreement with its weak physiological activity (Kutschmar et al., 2009). PSK binding is highest at low ionic strength and has an acidic pH optimum (Matsubayashi and Sakagami, 1999) in agreement with binding of the PSK ligand to the LRR domain of PSK receptors in the apoplast. Binding at pH 5.0 is about 3-fold higher than at pH 6.0, suggesting that cell wall acidification may promote PSK binding to its receptor and thereby enhance PSK signalling.

### The PSK receptor is a calmodulin-binding protein with dual activities as a kinase and guanylate cyclase

The extracellular LRR of PSK receptors is connected to the cytosolic Ser/Thr protein kinase by a single transmembrane helix followed by a juxtamembrane region (Matsubayashi et al., 2002) (Fig. 2). The cytosolic part of the receptor is a designated Ser/Thr kinase based on the 12 conserved subdomains of protein kinases (Kwezi et al., 2011). PSK receptors conform to the RD type of kinases, which have an activation loop (Kwezi et al., 2011). In the kinase-inactive state of most RD kinases the conformation of the activation loop prevents either substrate binding or phosphotransfer to the substrate (Johnson et al., 1996). Phosphorylation of the activation loop relieves this inhibition, resulting in kinase activation. Autophosphorylation of the soluble PSK receptor kinase suggests that the kinase may be regulated by phosphorylation of the activation loop (Hartmann et al., 2014). The recombinantly expressed cytoplasmic portion of the Arabidopsis receptor AtPSKR1 is an active kinase and has autophosphorylation as well as transphosphorylation activity (Kwezi et al., 2011; Hartmann et al., 2013). In addition, the PSK receptor has a designated guanylate cyclase (GC) catalytic centre within kinase subdomain IX (Kwezi et al., 2011), and Arabidopsis AtPSKR1 was shown to be a functional GC that produces cGMP in vitro when ectopically expressed as a soluble protein. The brassinosteroid receptor BR11 was likewise assigned GC activity (Kwezi et al., 2007; Wong and Gehring, 2013). However, using crystal structure analysis and enzyme assays of truncated soluble BR11 kinase, Bojar et al. (2014) came to the conclusion that BR11 is incapable of synthesizing cGMP, leaving the question as to whether or not BR11 produces cGMP a matter of debate. Plant protoplasts overexpressing full-length AtPSKR1 produce elevated levels of cGMP that are further increased in the presence of 0.1 μM PSK, but not when unsulfated PSK peptide is added. GC activity hence appears to be a property of the native receptor and is induced by PSK. The kinase accepts ATP and GTP as a co-substrate. The GC, however, produces cGMP but not cAMP, revealing specificity for GTP as a substrate.

**Arabidopsis** AtPSKR1 is a calmodulin-binding protein with the calmodulin-binding site located within kinase subdomain VIa (Hartmann et al., 2014). Binding of all four calmodulin isoforms of *Arabidopsis* occurs at this site. Calmodulin-like proteins do not bind at all or with low affinity. Calmodulin binding is abolished when a conserved tryptophan residue in the binding region is mutated to a serine (Hartmann et al., 2014). Moreover, plants expressing AtPSKR1\textsuperscript{W318S} in the Atpskr1-3 Atpskr2-I receptor knockout background have even shorter roots and smaller shoots as compared to the receptor null mutant, indicating, first, that the growth-promoting activity of the PSK receptor is regulated by calcium/calmodulin and, second, that the inactive receptor protein interferes with another growth-promoting signal pathway, probably at the level of protein interaction. In vitro, AtPSKR1 exhibits kinase and GC activities in the absence of calmodulin. Site-directed mutations of AtPSKR1 revealed that long-term plant growth promotion is dependent on AtPSKR1 kinase activity and on calmodulin binding (Hartmann et al., 2014). The contribution of the GC activity to defined PSK responses has yet to be described.

In the absence of calmodulin, there is ~2-fold promotion of GC activity of PSKR1 by ≥1 μM Ca\textsuperscript{2+}, and inhibition of kinase activity of PSKR1 by ≥100 mM Ca\textsuperscript{2+} has been reported to occur in vitro (Freihat et al., 2014). The PSK receptor does not harbour EF hand motifs for specific binding of Ca\textsuperscript{2+} (McCormack et al., 2005) and is therefore not a calcium sensor per se. It has yet to be clarified how Ca\textsuperscript{2+} alters PSK receptor activities in the absence of calmodulin.

### Table 2. Characteristics of PSK binding sites in rice, carrot, and Arabidopsis

<table>
<thead>
<tr>
<th>Receptor site</th>
<th>Ligand</th>
<th>High affinity</th>
<th>Low affinity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice plasma membrane-enriched</td>
<td>(^3\text{H})-PSK</td>
<td>(K_0 = 1.4) nM</td>
<td>(K_0 = 27) nM</td>
<td>1</td>
</tr>
<tr>
<td>Rice cells</td>
<td>(^3\text{H})-PSK</td>
<td>(K_0 = 1) nM</td>
<td>(K_0 = 100) nM</td>
<td>2</td>
</tr>
<tr>
<td>Rice plasma membrane-enriched</td>
<td>(^3\text{H})-PSK</td>
<td>(K_0 = 5) nM</td>
<td>(K_0 = 100) nM</td>
<td>3</td>
</tr>
<tr>
<td>Carrot microsomes</td>
<td>(^3\text{H})-PSK</td>
<td>(K_0 = 4.2) nM</td>
<td>(K_0 = 7.7) nM</td>
<td>4</td>
</tr>
<tr>
<td>Arabidopsis microsomes</td>
<td>(^3\text{H})-PSK</td>
<td>(K_0 = 7.7) nM</td>
<td>(K_0 = 1) nM</td>
<td>5</td>
</tr>
</tbody>
</table>

References: (1) Matsubayashi and Sakagami, 1999; (2) Matsubayashi et al., 1997; (3) Matsubayashi and Sakagami, 2000; (4) Matsubayashi et al., 2002; (5) Matsubayashi et al., 2006.
The sequence of events that includes receptor autophosphorylation and transphosphorylation, cytosolic calcium elevation, calmodulin binding, and cGMP production in situ has not been elucidated. It is conceivable that the two signal outputs, phosphorylation and cGMP synthesis, trigger unique downstream responses. One or both activities may also feed back on receptor activity. The dual kinase/GC activity of the PSK receptor is not unique in the plant kingdom. A similar protein structure with two enzymatic activities was demonstrated for the brassinosteroid receptor BR11 (Kwezi et al., 2007) and for the wall associated-like kinase 10 (Meier et al., 2010), and is predicted for additional kinases (Kwezi et al., 2007). Unraveling receptor characteristics and receptor regulation is an important part of elucidating the PSK signal pathway. To date, neither phosphorylation substrates, nor cGMP-regulated proteins, transcription factors, or gene targets that mediate a physiological response have been identified.

**PSK promotes growth**

Plant cells that are kept in liquid culture require auxin, cytokinin, and a minimum density to proliferate. Addition of nanomolar concentrations of PSK to the culture medium overcomes growth inhibition and induces cell proliferation of low-density cell cultures of asparagus and rice (Matsubayashi and Sakagami, 1996; Matsubayashi et al., 1997). However, in the absence of auxin and cytokinin PSK does not promote cell proliferation (Matsubayashi et al., 1999), suggesting that PSK is a quorum signal that renders cells permissive to induction of cell division by cytokinin and auxin in undifferentiated cultured cells.

The root apical meristem is sustained by slowly dividing QC cells that are surrounded by faster dividing stem cells. These continuously produce founder cells that generate the lateral and apical root cap and the different tissues of the root proper (Stahl and Simon, 2005). The AP2-type transcription factor gene ERF115 is expressed in QC cells (Heyman et al., 2013). Hydroxyurea, which blocks synthesis of deoxynucleotides (Conia et al., 1990; Juul et al., 2010) and hence G1/S phase progression, reduces the number of ERF115-GUS QC cells, suggesting that ERF115 is expressed in S or G2/M phase. The ERF115 protein is degraded by the APC\(^{CC32A2}\) complex, which is active during the G1 phase of the cell cycle (Heyman et al., 2013). ERF115 protein binds to the promoter of the PSK precursor gene AtPSK5 and transcriptionally activates PSK5 expression. Overexpression of ERF115 promotes QC cell division in an AtPSKR1-dependent manner, indicating that PSK signalling occurs in the QC. tspst-1 seedlings that are deficient in peptide sulfonylation activity have short roots. Their root apical meristem has excess QC cells and shows premature differentiation of root cap and root meristem cells (Matsuzaki et al., 2010). The defects in the root apical meristem are not overcome by PSK or PSY1, but PSK activity enhances the activity of another group of sulfated peptides, the RGFs. Interestingly, ERF115 ensures elevated QC cell division rates following death of the surrounding stem cells and thus restores root apical meristem function, probably due to its stress-induced expression (Heyman et al., 2013). AtPSKR1 enhances callus growth in Arabidopsis (Matsubayashi et al., 2006) and adventitious bud formation from calli of Antirrhinum majus; also, somatic embryogenesis in Cryptomeria japonica and carrot are promoted by PSK (Yang et al., 1999; Hanai et al., 2000; Igasaki et al., 2003). PSK furthermore maintains the growth of plants exposed to heat stress conditions (37°C) at night (Yamakawa et al., 1999).

Taken together, it is conceivable that PSK contributes to the formation and maintenance of the apical meristem under stress conditions such as wounding, in vitro culture, or heat stress. Incidentally, wounding increases expression of AtPSK4 in Arabidopsis and of ZePSK1 in Zinnia elegans (Matsubayashi et al., 2006); in addition, PSK suppresses expression of stress-related genes, including PR (pathogenesis-related) and PI (proteinase inhibitor) types, during the initial phase of tracheary element differentiation from isolated mesophyll cells in Zinnia (Motose et al., 2009), possibly to direct resources away from the wounding response towards the developmental trans-differentiation pathway.

In planta, PSK signalling promotes organ growth mainly via enhanced cell expansion. Arabidopsis seedlings that lack AtPSKR1 (Kutschmar et al., 2009; Stührwohldt et al., 2011) or both AtPSK1 and AtPSKR2 (Hartmann et al., 2013), have shorter hypocotyl and root cells than the wild type. Furthermore, mature root cells are longer in wild-type seedlings exposed to PSK as compared to untreated seedlings, suggesting that PSK triggers cell expansion during organ growth in planta. Similar to PSK, PSY1 promotes growth by increasing cell sizes in roots and shoots (Amano et al., 2007). The responses mediated by PSK and PSY1 are additive, suggestive of independent signalling pathways. Signalling of PSK in the epidermal cell layer suffices to promote root and shoot growth (Hartmann et al., 2013). Expression of AtPSKR1 in atrichoblast cell files not only promotes elongation of atrichoblasts but also elongation of trichoblast cells and of the entire root, indicating that PSK acts as a non-cell autonomous growth signal. Root elongation triggered by PSK in the tspst-1 background requires brassinosteroid synthesis and is dependent on the brassinosteroid receptor BR11. The brassinosteroid epi-brassinolide promotes root elongation growth at low concentrations (Missig et al., 2003) and may thus act as a mobile signal in the PSK response. Brassinosteroids also promote division of QC cells (Wildwater et al., 2005). In the QC, brassinolide enhances expression of ERF115 in a BR11-dependent manner, supporting the idea that it promotes QC cell division in response to PSK (Heyman et al., 2013). Taken together accumulating evidence suggests that the PSK and brassinosteroid signal pathways interact in elongating cells as well as in the QC.

**PSK signalling directs pollen tubes**

PSK precursor and receptor genes are expressed in a cell type- or tissue type-specific manner throughout the plant, including...
flowers, supporting a function of PSK signalling in reproduction. AtPSK2 in Arabidopsis (Stührwohldt et al., 2015) and ZmPSKI in maize (Lorbiecke et al., 2005) are expressed in pollen prior to and following germination. Pollen germination and tube growth in vitro are known to be density dependent, a phenomenon termed the pollen population effect. The germination rate of low-density tobacco pollen is promoted by PSK in a dose-dependent manner (Chen et al., 2000), and pollen tube elongation in Arabidopsis is dependent on PSK signalling (Stührwohldt et al., 2015). In planta, pollen tubes germinate on the stigma and traverse the transmitting tissue, until they grow along a funiculus that guides them to an embryo sac (Sauter, 2009; Dresselhaus et al., 2013). Lack of the PSK-sulfating enzyme TPST or knockout of PSK receptor genes results in reduced fertility (Stührwohldt et al., 2015). Loss of funicular pollen tube guidance was identified as the prime cause of infertility in tpst-1 and the Atpskr1-3 Atps2-1 receptor null mutant. The collective evidence indicates that PSK acts as a quorum signal in pollen, drives pollen tube growth, and provides a positional cue for pollen tubes at the crossroads from the transmitting tract to a funiculus, redirecting pollen tube growth towards an embryo sac.

PSK signalling integrates growth and plant immunity

In Arabidopsis, PSK signalling via AtPSKR1 contributes to resistance against the fungal necrotrophic pathogen Alternaria brassicicola (Igarashi et al., 2012; Mosher et al., 2013). Resistance is similarly mediated by PSY1 and its receptor PSY1R. psy1r plants show increased susceptibility to A. brassicicola. Knockout of both AtPSKR1 and PSY1R receptors causes additive disease symptoms. By contrast, PSK and PSY1 signalling attenuate the pathogen-associated molecular pattern (PAMP) response to the bacterial pathogen Pseudomonas syringae pv. tomato DC3000, causing enhanced resistance in Atpskr1-3 plants (Igarashi et al., 2012; Mosher et al., 2013). PSK and PSY1 likewise inhibit resistance to the pathogenic forma specialis of the fungus Fusarium oxysporum that causes wilting disease (Shen and Diener, 2013). Again, the signal pathways of PSK and PSY1 act independently and double receptor mutants display additive effects. The antagonistic effects of sulfated peptides in different pathogen responses suggest that PSK and PSY1 balance these defence responses. A basal activity is observed even in the absence of a pathogen when expression of some genes, such as jasmonic acid-dependent defence genes, is promoted by PSK (Mosher et al., 2013). PAMP-related defence genes are promoted by PSK signalling in seedlings but not in plants (Igarashi et al., 2012), revealing another layer of regulation that links defence control via sulfated peptide signalling to defined developmental stages. Given that PSY1 and PSK also act as growth factors (Amano et al., 2007; Kutschmar et al., 2009; Stührwohldt et al., 2011; Hartmann et al., 2013) it appears that sulfated peptides differentially integrate immune responses with growth to meet the competing metabolic challenges that arise during plant development.

Outlook

The disulfated peptide hormone phytosulfokine is perceived by an LRR-RLK at the cell surface. Current evidence indicates that the intracellular kinase of the PSK receptor is subject to regulation by Ca2+/calmodulin binding and by (auto-) phosphorylation in addition or in response to PSK binding, and that it has more than one signal output, namely kinase activity and cGMP synthesis. Future work will have to elucidate when and how receptor regulation occurs and how this affects one or both enzyme activities. We also need to understand if kinase and GC activities control different downstream events, and finally how the receptor is inactivated. Signalling downstream of the receptor is not understood. Hence, the signal transduction pathway which predictably involves interacting proteins, kinases, phosphatases, and transcription factors awaits elucidation. Based on the expression of PSK precursor and receptor genes, it appears possible that PSK may have physiological functions other than those described so far, and these await discovery. Finally it seems that the PSK signalling pathway should interact with other hormone pathways, such as brassinosteroid signalling, and we have yet to decipher the molecular mechanisms of these crosstalks.

Funding

The author acknowledges support by the Deutsche Forschungsgemeinschaft.

References


Hartmann J, Stührwoldt N, Dahlke Rl, Sauter M. 2013. Phytosulfokine control of growth occurs in the epidermis, is likely non-cell autonomous and dependent on brassinosteroids. The Plant Journal 73, 579–590.


Phytosulfokine signalling | 5169