Receptor kinase signalling in plants and animals: distinct molecular systems with mechanistic similarities

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Plant genomes encode large numbers of receptor kinases that are structurally related to the tyrosine and serine/threonine families of receptor kinase found in animals. Here we describe recent advances in the characterisation of several of these plant receptor kinases at the molecular level including the identification of receptor complexes, small polypeptide ligands and cytosolic proteins involved in signal transduction and receptor down-regulation. Phylogenetic analysis indicates that plant receptor kinases have evolved independently of the receptor kinase families found in animals. This hypothesis is supported by functional studies which have revealed differences between receptors kinase signalling in plants and animals, particularly concerning their interactions with cytosolic proteins. Despite these dissimilarities, however, plant and animal receptor kinases share many common features such as their single membrane-pass structure, their inclusion in membrane-associated complexes, the involvement of dimerisation and trans-autophosphorylation in receptor activation and the existence of inhibitors and phosphatases that down-regulate receptor activity. These points of convergence may represent features that are essential for a functional receptor kinase signalling system.

Addresses

Reproduction et Développement des Plantes, UMR 5667 CNRS-INRA-ENSL-UCBL, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France

Tel: 33 4 72 72 86 11

Fax: 33 4 72 72 86 00

e-mail: Mark.Cock@ens-lyon.fr

Abbreviations

EGFR	epidermal growth factor receptor
KAPP	kinase associated protein phosphatase
LRR	leucine-rich repeat
PRK	plant receptor kinase
RTK	receptor tyrosine kinase
RSK	receptor serine/threonine kinase
***S* locus**	self-incompatibility locus

Introduction - the receptor kinase gene superfamilies in plants and animals

One of the surprising observations that has recently come out of genome sequencing projects is that plant genomes are predicted to encode large numbers of receptor kinases with a eukaryotic-type kinase domain. *Arabidopsis thaliana*, for example, possesses 417 genes of this type [1**]. Until recently, these genes have been referred to as receptor-like kinases [2] but, based on the recent identification of several ligands that bind specifically to receptors of this family (see below), here we propose to use the term plant receptor kinase (PRK).

The structure of PRKs, which consist of an extracellular domain, a single membrane spanning domain and a cytosolic kinase domain, resembles those of the receptor tyrosine kinases (RTKs) and serine/threonine receptor kinases of the TGFβR family (RSKs) in metazoans. However, although PRKs are phylogenetically more closely related to these two families of animal receptor kinase than they are to other

kinase families, each of these three classes of receptor kinase can be grouped into a distinct, monophyletic family ([1**]; Figure 1). Moreover, so far PRKs have been found exclusively in plants whereas RTKs and RSKs have been found almost exclusively in the metazoan lineage and no receptor kinases belonging to these families have been found in the genomes of unicellular eukaryotes such as yeast [3]. Taken together, these data suggest that receptor kinases have evolved independently in the two kingdoms, their appearance during evolution perhaps coinciding with the acquisition of muticellularity. This would be consistent with the currently accepted hypothesis that plants and animals have evolved multicellularity independently from distinct unicellular ancestors [4].

Remarkably, considering the evolutionary history of receptor kinases in plants and animals, recent studies suggest that they function, to a certain extent, in an analogous manner at the biochemical level, indicating convergent evolution. The aim of this review is to describe recent advances in the characterisation of PRK function and to compare these receptor kinases with their counterparts in animals.

Structures and functions of plant receptor kinases

A common feature of the receptor kinase families in plants and animals is that, whilst the kinase domains are homologous, the extracellular domains can be highly diverse indicating that different protein domains have been recruited to function as extracellular domains in each family. The PRK superfamily includes receptors with more than 20 structurally distinct extracellular domains, the most common including domains with leucine-rich repeats (LRRs), S domains (homologous to the *S* [self-incompatibility] locus glycoprotein), domains with epidermal growth factor repeats and lectin domains [1**,5].

Functional analyses, using both mutagenesis and transgenic approaches, have shown that PRKs are involved in the regulation of a wide range of developmental processes. One of the first PRKs to be identified, the *S* locus receptor kinase (SRK), functions as the female component of self-incompatibility in *Brassica*, permitting the recognition and rejection of self pollen on the stigmas of self-incompatible plants [6,7]. Another PRK, CLAVATA1 (CLV1), is involved in the maintenance of the apical meristem in *Arabidopsis* and is part of a regulatory feedback loop that also includes the homeodomain-containing transcription factor WUSCHEL [8**,9*,10*]. Other developmental processes regulated by PRKs include organ elongation (ERECTA), floral organ abscission (HAESA), epidermal cell specification (CRINKLY4), post-meiotic pollen development (PRK1), cell elongation (WAK1) and brassinosteroid signalling (BRI1) [5]. Other members of the PRK superfamily such as FLS2 (which is involved in the perception of the bacterial elicitor flagellin) and Xa21 play a role in the plant's defence response [11,12].

Ligands

The recent identification of several soluble, extracellular ligands represents an important advance in our understanding of PRK function. One of the best characterised of these molecules is the *S* locus cysteine-rich protein (SCR/SP11 [13*,14*]) which has been shown to interact directly with SRK *in vitro* [15**,16*]. Genetic data, including the highly polymorphic nature of *SCR*, its location at the *S* (self-incompatibility) locus and the fact that it confers a self-incompatible phenotype to pollen when expressed in transgenic plants, strongly support the contention that SCR is the functional ligand of SRK.

There is also good evidence that a secreted polypeptide (CLV3), a brassinosteroid (brassinolide) and a fragment of bacterial flagellin proteins are ligands for three additional PRKs: CLV1, BRI1 and FLS2, respectively. In all three cases it has been demonstrated that these receptors are necessary for the binding and perception of their putative ligands [12,17**,18*,19*]. CLV3, for example, binds to yeast cells that co-express CLV1 and CLV2 (a truncated PRK that lacks a kinase domain [20]) and genetic data indicate that CLV1, CLV2 and CLV3 function in the same signalling pathway to control meristem development [21,22]. Finally WAK1, a PRK that is strongly bound to the cell wall, interacts specifically with a glycine-rich extracellular protein, AtGRP-3 [23*]. There is evidence that AtGRP-3 has a signalling role that would be coherent with its acting as a ligand for Wak1.

Assuming some degree of concerted evolution between structurally related PRKs and their respective ligands [24], it is likely that the identification of the molecules described above will facilitate the identification of ligands for other PRKs. Interestingly, two large families of genes that share homology with *SCR* and *CLV3* have been identified recently in the *Arabidopsis* genome [25,26].

As is the case with receptor kinases in animals, PRK ligands are almost exclusively small, secreted proteins. Brassinolide, which is a steroid, is an exception. Membrane-bound receptors for steroids are also thought to exist in animals, where they have been proposed to mediate non-genomic responses to these molecules, but these receptors have not yet been cloned [27].

Receptor complexes

PRKs, like their animal counterparts, are associated with other proteins in complexes. CLV1, for example, is associated *in vivo* with two complexes of ~185 and ~450 kDa

[28**]. The ~185 kDa complex probably corresponds to a disulphide-linked CLV1/CLV2 heterodimer [20]. Formation of the ~450 kDa complex requires a functional *CLV3* gene indicating that this complex contains the activated form of the receptor [28**]. The ~450 kDa complex is thought to represent a dimer of the ~185 kDa CLV1/CLV2 complex plus CLV3 and additional recruited proteins including kinase-associated protein phosphatase (KAPP) and Rop, a Rho/Rac-GTPase-related protein ([28**], Figure 2b).

A similar situation has been described for WAK1 which has been shown, by immunoprecipitation, to associate with its putative ligand, AtGRP-3, and with KAPP [23*]. Two complexes of ~200 and ~500 kDa were identified, AtGRP-3 and KAPP being associated with the larger complex.

SRK is also associated with high molecular weight, non-covalently linked complexes *in vivo*. In the absence of pollination, SRK in the plasma membrane of the stigmatic papillar cells is in a basal, inactive state [30*]. In this basal state, SRK is associated with two complexes of ~161 and ~290 kDa ([29*], D Cabrillac and T Gaude, unpublished data). Recently, cross-linking experiments using ^{125}I-labelled SCR ligand identified two ligand-binding proteins of 120 and 65 kDa [16*]. The 120 kDA protein was SRK whilst the 65 kDa protein may have been either eSRK (a soluble, truncated form of SRK produced by alternative splicing [31]) or SLG (a secreted glycoprotein that is highly similar to the extracellular domain of SRK and which is encoded by a gene closely linked to *SRK* at the *S* locus). Neither eSRK nor SLG are associated with SRK in the absence of pollination ([29*], D Cabrillac and T Gaude, unpublished data) suggesting that eSRK and/or SLG is recruited to the activated receptor complex following self-pollination (Figure 2a).

Both the SRK and CLV1 receptor complexes include truncated, receptor-like proteins that are either soluble (eSRK/SLG) or membrane-anchored (CLV2). Interestingly, genetic evidence indicates that binding of flagellin requires the product of a second locus, *FLS1*, in addition to the LRR receptor kinase FLS2 [32]. The *FLS1* gene has not yet been cloned, but it will be interesting to see whether the flagellin receptor and/or other PRK receptor complexes also include truncated PRK receptors. Truncated forms of many animal receptor kinases have been described but, apart from a limited number of studies which indicate that they act as receptor antagonists [33,34,35], their functions *in vivo* are unclear.

Receptor activation and signal transduction

Receptor oligomerisation and autophosphorylation are critical steps in the activation of animal receptor kinases of both the RTK and the RSK families [36,37]. Recent studies indicate that PRKs also autophosphorylate in response to ligand binding both *in vitro* and *in vivo* [16*,19*,30*] and there is evidence that phosphorylation can occur *in trans* within receptor oligomers. Using epitope-tagged receptors, transphosphorylation has been demonstrated between SRK molecules *in vitro* in a membrane environment [29*]. Moreover, the induction of SRK autophosphorylation that is seen following addition of pollen coat proteins (which include the SRK ligand, SCR) can be mimicked by adding a bivalent antibody that recognises the N-terminal end of SRK [30*]. This suggests that SCR, which probably exists as a dimer *in vivo* [15**], activates SRK by creating bridges between two molecules of the receptor. Note also that the ~450 kDa CLV1 complex was only detected in the presence of CLV3 suggesting that activation of CLV1 also involves oligomerisation.

Interestingly, mutations that lead to loss of kinase activity in either CLV1 or FLS2 also result in a loss of ligand binding activity [12, 17**]. This phenomenon has not been described for animal receptor kinases and it may be symptomatic of a difference between the mechanisms of activation of receptor kinases in plants and animals. Note, however, that there is evidence that this requirement of kinase activity for ligand binding may not be a general feature of all PRKs [15**].

At present, the only protein that has been shown to be a component of a signal transduction pathway downstream of a PRK is Arm repeat containing 1 (ARC1, Figure 2a). Suppression of ARC1 expression in transgenic *Brassica napus* plants causes a substantial weakening of the self-incompatibility response mediated by SRK [38*]. Rop, which is associated with the ~450 kDa CLV1 receptor complex, is also thought to be involved in downstream signalling. Plants lack a clear orthologue of Ras and it has been suggested that the related Rop protein activates a downstream MAPK cascade that negatively regulates *WUSCHEL* expression in the meristem ([39]; compare Figure 2b and d). Several additional, potential signal transduction components have been identified recently using protein-protein interaction, genetic and proteomic approaches [40,41,42].

Fine control of receptor kinase signalling

In animals, receptor kinases are highly regulated. For example, several mechanisms exist to down-regulate receptor kinase activity including kinase inhibitors, phosphatases and turnover via endocytosis. These processes are only starting to be investigated in plants but some interesting parallels can already be drawn.

In the absence of its ligand, constitutive activation of SRK is prevented by the thioredoxin-h THL1 (and probably also THL2) which binds to a site on the cytosolic

side of the transmembrane domain that includes a conserved cysteine residue [30*,43]. This situation is reminiscent of the action of FKBP12 which binds to the GS domain of TGFβR-I preventing phosphorylation of this receptor by its partner, TGFβR-II [44] (compare Figure 2a and c). THL1 and FKBP12 are unrelated at the sequence level indicating that the two inhibition systems have evolved independently. Interestingly, although thioredoxin has not been shown to interact with animal receptor kinases, it has been reported to inhibit murine p38 MAP kinase [45], suggesting an ancient origin for this type of regulation.

Dephosphorylation by phosphatases is a process that plays an important role in the down-regulation of receptor kinases in both plants and animals. KAPP has been shown to associate with a number of PRKs including CLV1 and FLS2 [18*,23*,46,47,48,49]. KAPP is phosphorylated by CLV1 and dephosphorylates the kinase domain of this receptor *in vitro* [47,48]. Experiments in which KAPP expression has been manipulated in transgenic plants indicate that KAPP is a negative regulator of both CLV1 and FLS2 [18*,47,48] and it has been proposed that KAPP has a general role in the down-regulation of a large spectrum of PRKs (Figure 2a, b). In animals, phosphatases such as SHP-1 also have a general role in the down-regulation of receptor kinase activity whilst others, such as SHP-2, are components of signal transduction pathways [50] (Figure 2d).

Down-regulation of RTKs following ligand binding involves endocytosis and receptor recycling or degradation. Polyubiquitylation, catalysed by the RING finger protein Cbl acting as a ubiquitin protein ligase, stimulates endocytosis and down-regulation of the epidermal growth factor receptor (EGFR). Interestingly, Cbl, shares several features with the SRK interacting protein, ARC1 (Figure 2a and d). Both Cbl and ARC1 have structures that are characteristic of adapter proteins, with multiple

protein-protein interaction domains, and both proteins interact only with the phosphorylated state of the kinase domains of their respective receptor interaction partners. Moreover, ARC1 possesses a U box [51], a domain that is structurally and functionally related to the RING finger domain [52]. The *Arabidopsis* genome contains 19 genes with a structure similar to ARC1 [51]. Might ARC1 or other members of this family mediate ubiquitylation of PRKs? This may be difficult to reconcile with the evidence that ARC1 acts as a positive regulator of SRK signalling but note that Cbl has been shown to have both negative and positive roles in signal transduction [53].

Down-regulation of animal receptor kinases by endocytosis is a slow process taking more than 30 minutes but this process is complemented by more rapid mechanisms involving several cytosolic proteins. One such protein is calmodulin, which binds to both EGFR and the insulin receptor and, at least in the case of EGFR, acts as an inhibitor [54]. Recently, we have shown that calmodulin binds to the kinase domains of several PRKs *in vitro* but the physiological role of this interaction remains to be elucidated (V.V. and J.M.C., unpublished results).

Conclusion

Considerable progress has been made in understanding the molecular mechanism of receptor kinase signalling in plants in recent years. One conclusion that can be drawn from these studies is that there are clear differences between receptor kinase signalling in plants and animals. For example, apart from calmodulin, none of the proteins that interact with PRKs are closely homologous to animal receptor kinase interacting proteins. These differences support the hypothesis that receptor kinases have evolved independently in the two kingdoms [1**].

Despite these differences between plant and animal receptor kinases, several basic features are shared such as receptor dimerisation, the existence of receptor complexes and certain mechanisms of downregulation. Given the distant relationship between receptor kinases in the two kingdoms, these similarities are of particular interest because they may represent convergently evolved solutions to the same fundamental molecular problems. This is particularly true when similar functions are mediated by unrelated molecules, for example if the inhibition of SRK by thioredoxin is compared with the inhibition of TGFβR by FKBP12. With the current acceleration in the pace at which PRKs are being characterised at the molecular level, comparisons of this kind, between plant and animal receptors, will become increasingly informative. In this context, it will be particularly interesting to address new questions that are emerging from studies on animal receptors in plant systems, such as the relationships between ligand binding, dimerisation and receptor activation [55,56*], the role of receptor localisation in signalling and the mechanism of turnover of activated receptor complexes [57].

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Figure 1

Model for the evolution of the receptor kinase superfamily based on a comparison of kinase domain sequences [1**]. Dark colours represent receptor kinase lineages, light colours represent related (homologous), cytosolic kinases. The arrowhead indicates the point at which the plant and animal lineages diverged.

Figure 2

Schema showing some of the features of inactive and ligand activated receptor kinase complexes in plants and animals. The colours highlight similarities between plant and animal receptor kinase complexes such as basal-state inhibitors (orange), phosphatases, (green), small G proteins (grey) and RING finger/U-box proteins (red). Association of KAPP and Rop with the plasma membrane has not been demonstrated experimentally. Red arrows indicate phosphorylation (P) and/or activation; red bars indicate dephosphorylation and/or inhibition. ARC1 possesses an ARM repeat region (A), a U-box (U) and a leucine zipper (Z); Cbl has a proline-rich domain (PR), a RING finger domain (R) and a TKB domain.

Keywords

Dimerisation, ligand, phosphatase, phosphorylation, plant receptor kinase, signal transduction