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Dividing maize tissues show preferential expression of two novel receptor-like cytoplasmic protein kinases

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Abstract

Two novel members of the receptor-like protein kinase (RLK) family were isolated from maize embryos by homology to ZmPK1, the first RLK identified in the plant kingdom. *PK8-a* and *PK8-b* share 88% sequence identity and code for deduced proteins with molecular masses of 57.3 and 56.6 K, respectively. The proteins contain a trans-membrane domain close to the N-terminus and a kinase domain at the C-terminal end. Despite the absence of an extracellular domain they phylogenetically belong to the RLK family where they fall into one of the receptor-like cytoplasmic kinase (RLCK) clades. The RT-PCR and in situ expression profiles reveal an ubiquitous expression with a preference for young, actively dividing tissues and suggest a role in a particular phase of the cell cycle. PK8-a and PK8-b are likely functionally redundant because insertional mutants of *PK8-a* did not exhibit any major phenotype despite a strong reduction in *PK8-a* transcript levels.

Keywords: Cell cycle; Development; Receptor-like cytoplasmic kinase (RLCK); Maize; *Zea mays*

1. Introduction

The dramatic increase in the number of genes coding for receptor-like kinases (RLK) in land plants is a striking example for major differences in the regulatory systems used by plants and animals [1]. They constitute over 2% of the *Arabidopsis* proteome lending support to the idea that plants have evolved their own pathways of signal transduction [2]. RLKs are part of the protein kinase super-family that regroups enzymes with the capacity to phosphorylate one or several amino acid side chains in a target protein. Depending on the type of residue phosphorylated the enzymes are classed as Tyr kinases, Ser/Thr kinases or His kinases. While animal receptor tyrosine kinases (RTK) phosphorylate essentially tyrosine residues, plant receptor-like kinases (RLK) phosporylate almost exclusively serine and/or threonine residues [3]. The phosphorylation allows an extremely rapid increase or decrease in the functional activity of the target protein. It is reversible depending on the relative abundance of protein kinase and counteracting phosphatases in the cell. In addition to the C-terminal kinase domaine the receptor kinase family is characterised by a N-terminal, ligand-binding extracellular domain and a single membrane-spanning domain [4].

The RLK family is not the only protein kinase family that shows substantial differences in gene number or organisation between animals and plants. For example, calcium signalling is mainly based on direct binding of the calcium ion via calcium-dependent protein kinases (CDPK) in plants rather than on indirect binding via calmodulin and calcium/calmodulin-dependent protein kinases (CaMK) as it is the case in animals [5]. Differences are less pronounced in the

Abbreviations: LRR, leucine rich repeat; PK, protein kinase; RLCK, receptor-like cytoplasmic kinase; RLK, receptor-like kinase

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case of the cyclin-dependent kinases (CDK) involved in cell cycle regulation. While a plant specific class exists, numerous other plant CDKs have animal counterparts [6]. In the case of mitogen-activated protein kinases (MAPK) regulating cell growth and death, differentiation and stress responses the same type of genes are found in animals and plants, although gene number is roughly tripled in *Arabidopsis* as compared to humans [7]. Finally, relatives of the His kinases involved in plant hormone, stress and light signalling have not been found in animals but are present in prokaryotes and lower eukaryotes [8]. However, the expansion of the RLK family from less than 5 genes in mammals to over 600 genes in *Arabidopsis* [3] and over 1100 genes in rice [9] is unprecedented by its extent. The determination of the precise function of these proteins and the search for the corresponding ligands will be one of the major challenges for plant biologists in upcoming years.

The biological functions of plant RLKs are quite diverse reflecting the multitude of extracellular domains linked to the conserved trans-membrane and protein kinase domains within a single polypeptide. They can be grouped into two broad categories namely the control of plant growth and development or the interaction with pathogens or symbionts [3,10]. Examples for the first category are Clavata1 (CLV1) controlling meristem development [11], S-receptor kinase (SRK) involved in self-incompatibility [12] or brassinosteroid insensitive1 (BRI1) mediating brassinosteroid signalling [13]. For these three proteins the corresponding ligands have been identified: the small protein CLV3 for CLV1 [14,15], the small pollen protein SRC for SRK [16] and plant hormones of the brassinosteroid family for BRI1 [17]. The most active compound is brassinolide and direct interaction between a brassinolide precursor and BRI has recently been demonstrated [18]. Examples for the second category are FLS2 involved in the general defence against bacteria in *Arabidopsis* [19], Xa21 mediating resistance to bacterial leaf blight in rice [20], SR160 in systemin signalling in tomato [21] or nodulation receptor kinase (NORK) involved in early steps of the symbiosis between *Rhizobium* and alfalfa [22,23]. The ligand is clearly identified in the case of FLS2 that binds flagellin, a structural protein showing a high conservation of its N-terminus over a broad range of bacteria [24].

Since the discovery of the first plant RLK in maize [25] several other maize RLKs have been described. The best characterised is Crinkly4, a tumour necrosis factor receptor-like protein kinase involved in the control of leaf development and the specification of aleurone cell fate in the developing kernel [26]. While the function of Crinkly4 has been established by mutant analysis and molecular characterisation, in other cases functions have been suggested merely based on restricted gene expression patterns. Two of the three LRR-type LTK proteins may play a role in endosperm development [27], one of the two LRR-type SERK proteins is thought to be involved in reproductive events [28] and the LRR-type PRK1 is likely important for pollen development [29]. In addition, a maize atypical receptor kinase (MARK) has been characterised that does not exhibit any kinase activity in vitro but strongly stimulates the kinase activity of MIK, a member of the MAPKKKK family. The preferential expression in proliferating tissues of the embryo implies a role in embryo development [30]. Three maize histidine protein kinases (ZmHK) involved in cytokinin signal transduction are fully fledged receptor kinases but are generally not considered as part of the RLK family but rather of the His-Asp phospho-relay family [31]. In addition the maize orthologue of the *Arabidopsis* kinase associated protein phosphatase (KAPP) has been isolated and characterised suggesting conservation of RLK signalling mechanisms in monocots and dicots [32].

We present here the isolation and characterisation of PK8-a and PK8-b, two novel RLKs from maize. Despite the absence of an extracellular domain they phylogenetically belong to the RLK family where they fall into one of the receptor-like cytoplasmic kinase (RLCK) clades. The RT-PCR and in situ expression profiles reveal an ubiquitous expression with a preference for young, actively dividing tissues. Insertional mutants of *PK8-a* did not exhibit any major phenotype suggesting functional redundance with the closely related gene *PK8-b*.

2. Materials and methods

2.1. Plant material and growth conditions

Maize mutants of Pioneer Hibred's TUSC-collection [33] were propagated by backcrosses to lines A344 or A188 either in the green house with a 16 h illumination period (100 Wm-2) at 24/19 °C (day/night) or under field conditions at the ENS in Lyon (France).

Inbred line A188 [34] was used for the construction of cDNA libraries and expression profiles and hybrid DH5 × DH7 [35] for the construction of a genomic library. Both were grown in a growth chamber with a 16 h illumination period (100 Wm-2) at 24/19 °C (day/night) and 80% relative humidity. All tissues were harvested at mid-morning. The young leaf sample corresponds to the blade of the youngest leaf at the eight-leaf stage and the mature leaf sample to the blade of the fourth leaf from the bottom at flowering. Young roots were collected from 1-week-old seedlings germinated in vitro on filter paper and mature roots from plants grown in sand at the eight-leaf stage. Immature tassels had emerged from the covering leaves but had not separated the branches, while mature tassels deployed stamens on the majority of branches. For the harvest of pollen the tassel was shaken to eliminate ancient pollen, covered for 4 h with a paper bag and shaken again to collect fresh pollen. The visible parts of silks were cut at an apparent length of 8 cm from protected ears. For the stem sample the part between the root and the first leaf was cut at the eight-leaf stage and the sheaths of the leaves removed. Immature ears of 4–6 cm were harvested before the emergence of the first silk;