KINKY POLLEN and POKY POLLEN TUBE are Two Novel Genes Required for Tip Growth and Duplicated in the Arabidopsis Genome

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Screening of male gametophytic mutants from the Versailles collection of T-DNA transformants allowed us to isolate and characterize two novel genes: KINKY POLLEN (KIP) and POKY POLLEN TUBE (POK), which are required for correct tip growth in Arabidopsis thaliana. As KIP and POK are expressed in all plant tissues, though to a higher level in pollen and roots, their roles may not be restricted to tip growth only, but might extend to more general elongation mechanisms. Both genes are duplicated in the Arabidopsis genome. Specific roles for each duplicate, indicated by mutant phenotypes, will be discussed. Moreover, KIP and POK proteins have putative orthologs in all eukaryotes investigated, suggesting that they may be crucial proteins required for correct polar growth in all eukaryotic species.

Key words: Arabidopsis thaliana, pollen tube, tip growth, duplication.

INTRODUCTION

Due to its central role of the male gametophyte in the plant life cycle, its development has been studied and described well for many years (Bedinger et al., 1994). To gain insight into the molecular mechanisms involved in this process, we and others (e.g., Howden et al., 1998) have undertaken genetic screening of the Arabidopsis thaliana T-DNA transformant collection to isolate male gametophytic mutations based on the 1:1 segregation ratio of the kanamycin resistance marker gene carried by T-DNA in selfing progeny (Bonhomme et al., 1998a,b). Two such male gametophytic mutants, kinky pollen (kip) and poky pollen tube (pok), both affected in pollen tube growth, have been identified. Pollen tube elongation is a key step of pollen development and fertilization, since it allows the transport of the male gametes to their female counterparts. The pollen tube is a cylindrical structure which elongates following a very efficient and polarized mode of growth, tip growth (Hepler et al., 2001), as a result of apical exocytosis of Golgi vesicles containing parietal precursors. Two intracellular structures are supposed to be particularly important to this process: the cytoskeleton and the Golgi apparatus (Geitmann and Emons, 2000). Tip growth is a mechanism shared by only a limited set of cell types in eukaryotes: pollen tubes and root hairs of plants, fungal hyphae, and animal axons (Palanivelu and Preuss, 2000). Although the molecular players involved in this process are beginning to be highlighted (Hepler et al., 2001), KIP and POK are the first novel proteins described so far that play an important role in the course of tip growth and for which corresponding genes have been cloned. Here we analyze the structure and possible origins and functions of these genes.

MATERIALS AND METHODS

The Ttd (T-DNA transmission defect) lines of A. thaliana (L.) were isolated from the Versailles collection of T-DNA insertion mutants and cultivated as described in Bonhomme et al. (1998a). Pollen grains from young open flowers were cultured as described by Procissi et al. (2001), and Hodgkin (1983).
RESULTS

KIP AND POK ARE TWO MALE GAMETOPHYTIC MUTATIONS AFFECTING TIP GROWTH

Transmission defects through the pollen for three allelic kip mutant lines (Ttd26, Ttd34 and Ttd42) and the single pok mutant line (Ttd8) have been described in Bonhomme et al. (1998a) and Procissi et al. (2001).

In vitro pollen germination experiments (Fig. 1) show that both mutations affect pollen tube elongation. For kip hemizygous mutants, 50% of the pollen tubes are twisted and sometimes branched. The kinky shape of kip pollen tubes results from growth arrests and reorientations of the growth axis, as shown by Procissi et al. (submitted). The penetrance of the mutation differs between the three kip alleles, Ttd34 being a null one. Shorter and thicker root hairs are also observed in kip homozygous lines (not shown), confirming the involvement of the KIP gene in tip growth.

When cultivated in vitro, pollen from the hemizygous pok mutant have 50% of the pollen tubes shortened, even after 12 h culture. This and previously published T-DNA transmission data (Bonhomme et al., 1998b) suggest that tip growth either is precociously arrested or is severely slowed. To investigate the potential root hair mutant phenotype, pok homozygous mutants were searched, without success, suggesting that the homozygous pok mutation leads to embryo lethality.

KIP AND POK ARE EXPRESSED IN ALL PLANT TISSUES

Study of the expression patterns of both genes (not shown) indicates that KIP and POK transcripts are expressed in all plant tissues at a low level; however, expression levels were higher in roots and anthers for the KIP gene, and in roots and flower buds for the POK gene.

BOTH KIP AND POK SEEM TO BE DUPLICATED IN THE ARABIDOPSIS GENOME

The KIP gene is located on chromosome V and transcribed in a 7.8 kb long fragment of mRNA exhibiting 66% identity with SABRE (SAB) cDNA. The SAB gene is located on chromosome I, and sab1 mutants have been described (Aeschbacher et al., 1995). While homozygous sab mutants show a global dwarf phenotype, no abnormal phenotype has been observed in tip-growing cells (pollen tubes or root hairs). The SAB gene is expressed in all tissues at a constant level, more or less similar to the basal level of KIP transcript expression (not shown).

The POK gene is located on chromosome I and is transcribed in a 2.2 kb mRNA fragment. Four kb downstream from the POK gene is another gene which we named P2, whose transcript exhibits 90% identity with the POK transcript. No P2 mutant is available so far, but RT-PCR experiments suggest that the P2 gene is expressed in all plant tissues, although at a very low level.

For both KIP/SAB and POK/P2 pairs, the intron/exon structures are highly conserved between duplicated genes (Fig. 2), with a strong identity between exon sequences, whereas intron sequences are totally divergent. KIP and SAB both contain 23 exons, sharing 44% to 80% identity. The POK and P2 exons (20 and 19, respectively; POK having an additional 5' exon) share 79% to 98% identity. On average, SAB introns are longer than KIP introns, whereas introns are shorter in P2 than in POK.

DEDUCED KIP AND POK PROTEINS ARE CONSERVED AMONG EUKARYOTES

Deduced KIP and POK proteins (2587 and 708 amino acids, respectively) have putative orthologs in many eukaryotic species, from yeast to human (Tab. 1). In each case, homologies are spread over the entire protein sequence. The function of the KIP...
protein is still unknown, but prediction software packages identify a signal peptide that could target it to the secretory pathway. The *Saccharomyces cerevisiae* putative ortholog of POK, Vps52p, has been shown to be involved in the recycling machinery of Golgi-resident proteins (Conibear and Stevens, 2000).

**DISCUSSION**

**kip** and **pok** are two male gametophytic mutations affecting tip growth. The sequences of KIP and POK proteins are conserved among numerous plant and animal species in which tip-growing cells have been described. This high conservation suggests a crucial function for both proteins. Both the location and function of the KIP protein remain to be elucidated, although a role in extracellular matrix organization can be hypothesized (unpublished results).

The role of its putative yeast ortholog, Vps52p, in Golgi vesicle recycling, suggests that the function of POK could extend to more general growth mechanisms and not be restricted to tip growth only. This idea is reinforced by different observations: (i) the POK gene is ubiquitously expressed, (ii) the POK protein is abundantly present in the root elongation zone (not shown), and (iii) the pok homozygous mutation is suspected to lead to embryo lethality. Thus, POK could be required for correct Golgi vesicle trafficking in any elongating or dividing cell.

Duplication of both the KIP and POK genes is not very surprising, since it is now thought that *Arabidopsis thaliana* must be a degenerate tetraploid, following a duplication of its whole genome 112 million years ago (Ku et al., 2000). As is the case for the POK and P2 genes, a large number (17%) of *Arabidopsis* genes are tandemly repeated, whereas other portions of the genome have been subsequently rearranged, leading to the separation of the duplicated genes, as for KIP and SAB (The Arabidopsis Genome Initiative, 2000; Blanc et al., 2000).

Genome evolution studies demonstrate that a gene duplicate can follow four fates (Lynch and Conery, 2000) for which examples are known in plant genomes: (i) true and complete redundancy is conserved between both duplicates (e.g., SHP1 and 2 genes of *Arabidopsis*) (Liljegren et al., 2000); (ii) nonfunctionalization: one copy is silenced by degenerative mutations, while the other maintains its initial function (e.g., TGG3 of *Arabidopsis*) (Zhang

| TABLE 1. Protein sequence comparisons between KIP, POK and their putative orthologs in few eukaryotic species |
|-------------------------------------------------|--------------------|-----------------|-----------------|--------------------|-----------------|
| Species                                         | KIP                | POK             |                 |                   |                 |
|                                                 | %identity | %similarity | Acc.number | %identity | %similarity | Acc.number |
| *Arabidopsis thaliana*:SAB                       | 57        | 76          | AAC49734     | -         | -           | -           |
| *A. thaliana*:P2                                 | -         | -           | -            | -         | -           | -           |
| *Saccharomyces cerevisiae*                       | 12        | 34          | AAB68087     | 23        | 43          | AAB649122 |
| *Caenorhabditis elegans*                         | 13        | 32          | CAB07193     | 26        | 47          | AAA68727 |
| *Drosophila melanogaster*                        | 13        | 34          | AAF47740     | 32        | 55          | AAF52254  |
| Homo sapiens                                    | 13        | 32          | BAA07891     | 34        | 57          | AAH32108  |

*a* corresponding to Vps52p

Fig. 2. Structure of KIP, SAB, POK and P2 genes. Even exons are colored light grey, uneven in dark grey, to facilitate comparison of duplicated gene structures. Arrows indicate T-DNA insertion positions for the different mutants available. sab2 is a mutant from the Versailles *Arabidopsis* T-DNA transformant collection (our unpublished data).
et al., 2000); (iii) neofunctionalization: one copy is preserved in its original fate while the second evolves to assume a new beneficial function and is thus preserved (e.g., chalcone synthase shift of function into stilbene synthase in *Antirrhinum*) (Durbin et al., 2000); and (iv) subfunctionalization: both copies are preserved and both of them fix complementary loss-of-function mutations (e.g., WER and GL1 genes of *Arabidopsis*) (Kellogg, 2001).

Considering both KIP/SAB and POK/P2 pairs, redundancy can clearly be dismissed, as the mutant phenotype can be observed when one gene of the pair is mutated. KIP and SAB genes might correspond to the neofunctionalization case, as it seems that one gene (SAB) is involved in diffuse growth processes, whereas the other one (KIP) became specialized for tip-growing cells. This is suggested by the KIP mutant phenotype and the KIP expression pattern. Concerning the POK/P2 duplication, the possibilities of neofunctionalization, subfunctionalization and non-functionalization (i.e., considering P2 as evolving to a pseudogene) can also be hypothesized. There are several examples of "young pseudogenes" that have conserved their exon/intron structure and are still expressed, though at a very low level (Ramos-Onsins and Aguadé, 1998; Zhang et al., 2000). We are now searching P2 mutant lines to discriminate between these hypotheses.

It is interesting that characterization of two independent male gametophytic mutations led us to two novel genes highly conserved among eukaryotes, both of which are involved in polarized growth and duplicated in *Arabidopsis*. Further study of both KIP and POK proteins should provide new insights about tip growth and more general elongation processes, and perhaps yield new clues to understanding the wide redundancy of the *Arabidopsis* genome.

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REFERENCES


