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RPE, a plant gene involved in early developmental steps of nematode feeding cells

Bruno Favery, Philippe Lecomte, Nathalie Gil, Nicole Bechtold¹, David Bouchez², Antoine Dalmasso and Pierre Abad³

INRA, Laboratoire de Biologie des Invertébrés, 123 bd F. Meilland, 06600 Antibes, ¹INRA, Laboratoire de Génétique et d'Amélioration des Plantes, Route de Saint Cyr, 78026 Versailles Cedex and ²INRA, Laboratoire de Biologie Cellulaire, Route de Saint Cyr, 78026 Versailles Cedex, France

³Corresponding author
e-mail: abad@antibes.inra.fr

Sedentary plant-parasitic nematodes are able to induce the redifferentiation of root cells into multinucleate nematode feeding sites (NFSs). We have isolated by promoter trapping an *Arabidopsis thaliana* gene that is essential for the early steps of NFS formation induced by the root-knot nematode *Meloidogyne incognita*. Its pattern of expression is similar to that of key regulators of the cell cycle, but it is not observed with the cyst nematode. Later in NFS development, this gene is induced by both root-knot and cyst nematodes. It encodes a protein similar to the D-ribulose-5-phosphate 3-epimerase (RPE) (EC 5.1.3.1), a key enzyme in the reductive Calvin cycle and the oxidative pentose phosphate pathway (OPPP). Quantitative RT-PCR showed the accumulation of RPE transcripts in potato, as in *Arabidopsis* NFS. Homozygous *rpe* plants have a germination mutant phenotype that can be rescued in dwarf plants on sucrose-supplemented medium. During root development, this gene is expressed in the meristems and initiation sites of lateral roots. These results suggest that the genetic control of NFSs and the first stages of meristem formation share common steps and confirms the previous cytological observations which indicate that root cells undergo metabolic reprogramming when they turn into NFSs.

Keywords: *Arabidopsis thaliana*/D-ribulose-5-phosphate 3-epimerase/pentose phosphate pathway/promoter trapping/sedentary endoparasitic nematodes

Introduction

Among plant pathogens, sedentary endoparasitic nematodes interact with their hosts in a most fascinating way. They are able to induce the redifferentiation of root cells into nematode feeding sites (NFSs). Nematode growth and reproduction depend on the establishment of these NFSs. Nematodes withdraw food from NFSs until the completion of their life cycle, causing a tremendous threat to crop production worldwide (Sasser and Freckman, 1987). It is not yet understood how these nematodes cause such alterations, but it is suspected that glandular secretions

injected into plant cells interact directly or indirectly with the plant nuclear genome (Hussey, 1989). Cytological observations have indicated that NFSs are multinucleated with an enlargement of the nucleus and the nucleolus. Compared with normal cells, NFSs also show an increase in cytoplasmic density, a loss of normal vacuolation and a proliferation of cell organelles. Another characteristic feature of these structures is the development of cell-wall ingrowths, typical of transfer cells (Jones, 1981). These cell-wall ingrowths increase the surface area of the associated membrane and thus facilitate the import of elaborated photosynthates, minerals and other metabolites. Depending on the nematode species, the initial feeding cell develops into either a syncytium (for cyst nematodes such as *Heterodera* spp. and *Globodera* spp.) or a system of giant cells (for the root-knot nematodes *Meloidogyne* spp.) (Jones, 1981). Syncytia result from cell fusions after cell-wall dissolutions between the initial cell on which the nematode starts feeding and an increasing number of neighboring cells. Up to 200 cells can be incorporated in a large syncytium. Conversely, giant cell formation is the result of repeated nuclear divisions of the initial feeding cell without cytokinesis (Huang, 1985). Each root-knot nematode triggers the development of five to seven giant cells, each containing as many as 100 nuclei, which have undergone vast endoreduplication (Wiggers *et al.*, 1990). Because *Meloidogyne* species can induce similar giant cells in several thousand host species, they probably interact with some fundamental key steps of the plant cell cycle (Niegel *et al.*, 1996). In addition, root-knot nematode development is accompanied by divisions of cortical cells around the NFS, giving rise to a typical root-knot or gall. These complex morphological and physiological changes during the establishment of NFSs are reflected by altered gene expression in affected root cells (Gheysen *et al.*, 1996; Williamson and Hussey, 1996). Approaches based on differential gene expression between healthy and infected roots have allowed the identification of cDNA clones with homology to several known plant defense genes (Niegel *et al.*, 1993, 1995; Lambert, 1995). In parasitized cells, there is upregulation of cDNAs homologous to a key component of the protein ubiquitination pathway (E_2 enzyme), a large subunit of RNA polymerase II, a Myb-type transcription factor and a plasmalemma H^+ -ATPase (Bird and Wilson, 1994). There is also an upregulation of a late embryogenesis-abundant protein (Van der Eycken *et al.*, 1996). Sequencing of differentially expressed genes and computer searching of molecular data banks might indicate a putative function for the products they encode. This approach, however, must be coupled with biochemical and physiological investigations if their actual function is to emerge. More direct evidence for the role of known plant genes in the establishment or maintenance of NFSs came from a variety of promoter-*gusA* fusion constructs

introduced into *Arabidopsis* and tobacco (Goddijn *et al.*, 1993; Niebel *et al.*, 1996). It has thereby been shown that the root-specific promoter *TobRB7* (Conkling *et al.*, 1990), which encodes a presumed water channel expressed in root meristematic and immature vascular cylinder regions, is reactivated in tobacco giant cells induced by *Meloidogyne incognita* (Yamamoto *et al.*, 1991; Opperman *et al.*, 1994a). Similarly, transcriptional activation of cell cycle markers such as the cyclin-dependent kinase *CDC2a* and the mitotic cyclin *CYC1A*t is observed during the early stages of NFS formation (Niebel *et al.*, 1996). Moreover, many other genes are downregulated in NFSs. For example, promoters of the bacterial nopaline synthase and the plant phenylalanine ammonia-lyase I gene, which are highly active in non-infected roots, are silenced within a few days of nematode infection (Goddijn *et al.*, 1993).

To identify new genes and obtain a more comprehensive view of the molecular mechanisms underlying the induction and maintenance of NFSs, a promoter-trapping strategy was developed, with a promoterless β -glucuronidase (GUS) construct being introduced randomly into the *Arabidopsis* genome via *Agrobacterium* T-DNA transformation (Kertbundit *et al.*, 1991; Topping *et al.*, 1991; Goddijn *et al.*, 1993). This 'tagging' approach has been used in several laboratories, and tagged lines have recently been identified. However, despite interesting expression patterns in NFSs, none of these T-DNA flanking regions have presented homology with known genes (Barthels *et al.*, 1997).

Here we report the molecular characterization of a strongly upregulated nematode-responsive gene in the early steps of giant cell formation induced by *M. incognita* in *Arabidopsis*. This gene encodes for the D-ribulose-5-phosphate 3-epimerase (RPE), an enzyme involved in the pentose phosphate pathway. This upregulation is also observed in syncytia induced by *Heterodera schachtii*, but at a lower level and later after nematode infection. Moreover, we demonstrated that this gene is similarly regulated in potato after infection by both cyst and root-knot nematodes. Finally, the implication of this enzyme in root apex and lateral root initiation sites indicates that the genetic control of NFSs and root formation share common steps and suggests that root cells undergo metabolic reprogramming when turning into NFSs.

Results

Characterization of the RPE-tagged line

To isolate molecularly tagged lines showing GUS induction or repression in galls, we screened a collection of T-DNA-tagged *Arabidopsis* lines (ecotype WS) obtained by *in planta* transformation (Bechtold *et al.*, 1993; Bouchez *et al.*, 1993). These lines were screened in a GUS assay 7 days after infection with *M. incognita*. Of 3000 T-DNA tagged lines tested, 25 transgenic lines showed increased GUS expression, and three transgenic lines showed a repressed GUS expression in the galls. In other tissues, specific GUS activity was detected in 7% of the transformants. None of the tagged lines showed GUS expression that was exclusively restricted to the galls. Expression was observed in other parts of the plants and was always found in the root apex. One of these lines, named RPE, showed early and strong GUS expression in the galls. It

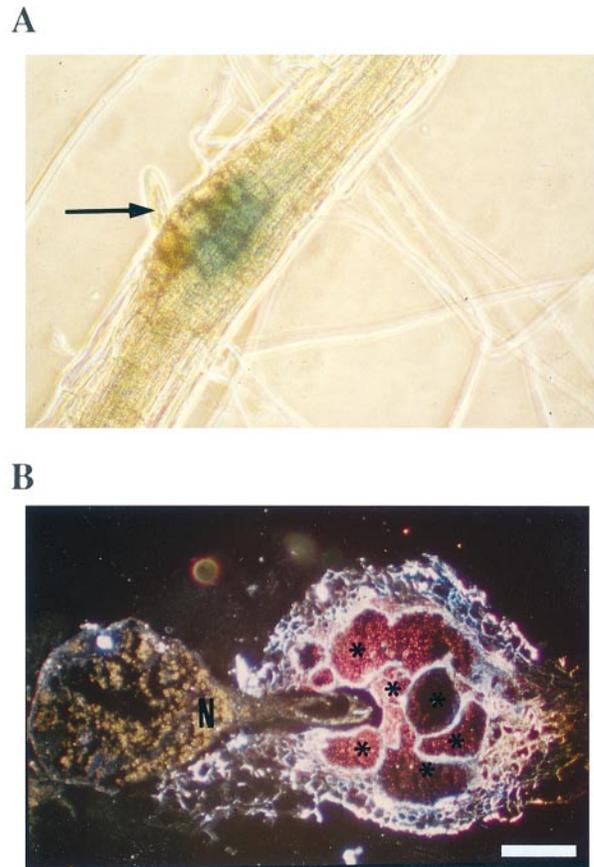


Fig. 1. GUS expression in galls of RPE-tagged line induced by *M. incognita*. (A) Localized GUS expression in root gall (arrow) 7 days after infection. (B) Cross-section of a gall 10 days after infection. Galls were excised from histochemical β -glucuronidase- (GUS) stained plants, fixed and cryosectioned. Sections, examined under dark-field light, clearly showed GUS expression (pink precipitate) limited to giant cells. The head of the feeding nematode (N) can be seen at the edge of the giant cells (*). Bar, 100 μ m.

presented an abnormal segregation (2:1 instead of 3:1) of the kanamycin marker carried by the T-DNA, suggesting mutant-impaired seed germination (embryo-lethal, seed viability or germination mutant). Of the 435 T2 seeds tested in bulk, 287 grew on kanamycin medium and displayed a normal phenotype compared with the wild type. This frequency fitted well with the hypothesis of the ratio of kanamycin-resistant (K^r) to sensitive (K^s) being 2:1 ($\chi^2 = 0.09$; $P > 0.05$), suggesting also that the T-DNA was inserted in one nuclear locus.

To analyze precisely at which stage of the interaction the GUS gene is activated, we infected the RPE line *in vitro*. Time-course experiments pointed out the presence of an early induced plant promoter that activates the transgene in young galls less than 3 days post-infection (d.p.i.), that is to say, 24–48 h after giant cell initiation (Jones, 1981; Wyss *et al.*, 1992). No GUS expression was detected at the penetration site (in the elongation zone) nor during nematode migration. The RPE line showed a strong GUS expression at 7 d.p.i. in galls induced by *M. incognita* (Figure 1A). This expression was maintained until sexual maturity of the females. To determine the localization of GUS expression, we made thin cryosections of the galls. Cross sections of 10-day-old galls clearly

showed GUS staining in the giant cells of the gall (Figure 1B). No significant GUS activity was seen in cortical cells surrounding the feeding cells. Similar results were obtained with other *Meloidogyne* species such as *M.javanica* and *M.hapla*, and with the beet cyst nematode *H.schachtii* Schmidt (data not shown). Nevertheless, the latter species induced GUS expression later in the feeding cell (15 d.p.i.). During plant development, the GUS gene was expressed in the root meristem and in part of the elongation zone, in which cells divide and expand (Figure 2A). GUS activity was also visible early in the lateral root primordia, before visible evidence of root formation (Figure 2B). High levels of expression were also observed in aerial parts of the plant such as flowers (Figure 2C). In mature embryos from dry seeds, GUS activity was observed through more-intense staining in the zone corresponding to the root apical meristem and in cotyledons (Figure 2D), young tissues with active DNA synthesis for cell polyploidization (Brown *et al.*, 1991).

To ascertain whether the T-DNA insertion was closely linked to the *rpe* mutation, we analyzed cosegregation of the mutant phenotype with the T-DNA insertion. Fifty T4 progenies resulting from each of the 40 selfed K^r T3 plants segregated with the expected ratios of 1/4 non-germinating seeds (mutant homozygous phenotype) and 3/4 germinating seeds of which 2/3 were K^r and 1/3 K^s (data not shown; $\chi^2 = 0.10$; $P > 0.05$). Thus there was no recombination between the *rpe* mutation and the T-DNA cassette. These results suggested that *rpe* mutation was recessive; it prevented germination when homozygous and was actually linked to the T-DNA.

Molecular cloning of the RPE gene

DNA hybridization analysis of RPE plants through internal fragments of the T-DNA, *GUS* (Figure 3A) and left border (data not shown), confirmed that the RPE line carries a single intact T-DNA insert. A 515 bp genomic DNA fragment adjacent to the right border of the T-DNA was isolated by kanamycin plasmid rescue with *Pst*I restriction sites (Bouchez *et al.*, 1996) (Figure 3B). Using an inverse PCR strategy (IPCR; Earp *et al.*, 1990) we cloned 657 bp of DNA flanking the left border (LB) of the T-DNA (*Bgl*II site from the IPCR primers T4 and T5) (Figure 3B). These two flanking sequences were used as probes on a Southern blot and indicated that the cloned fragments corresponded to a unique genomic fragment (data not shown). To analyze the insertion point of the T-DNA, we designed the oligonucleotides LA2 and RA2 (Figure 3B). Comparison of the nucleotide sequences of the transformed line with wild-type sequence revealed that a deletion of 77 bp and an insertion of a 5 bp filler sequence at the end of the right border resulted from the insertion of the T-DNA. In addition, typical deletions in the 24 bp repeat of the left and right borders were observed; they resulted in the presence of only one nucleotide from the left border at the insertion junction (Gheysen *et al.*, 1987; Mayerhofer *et al.*, 1991).

Sequence analysis of the RPE cDNA

The *RPE* cDNA was cloned by rapid amplification of cDNA ends (5' and 3' RACE; Frohman *et al.*, 1988) by using poly(A)⁺ RNAs from the wild-type *Arabidopsis thaliana* ecotype WS. Only one class of transcripts was

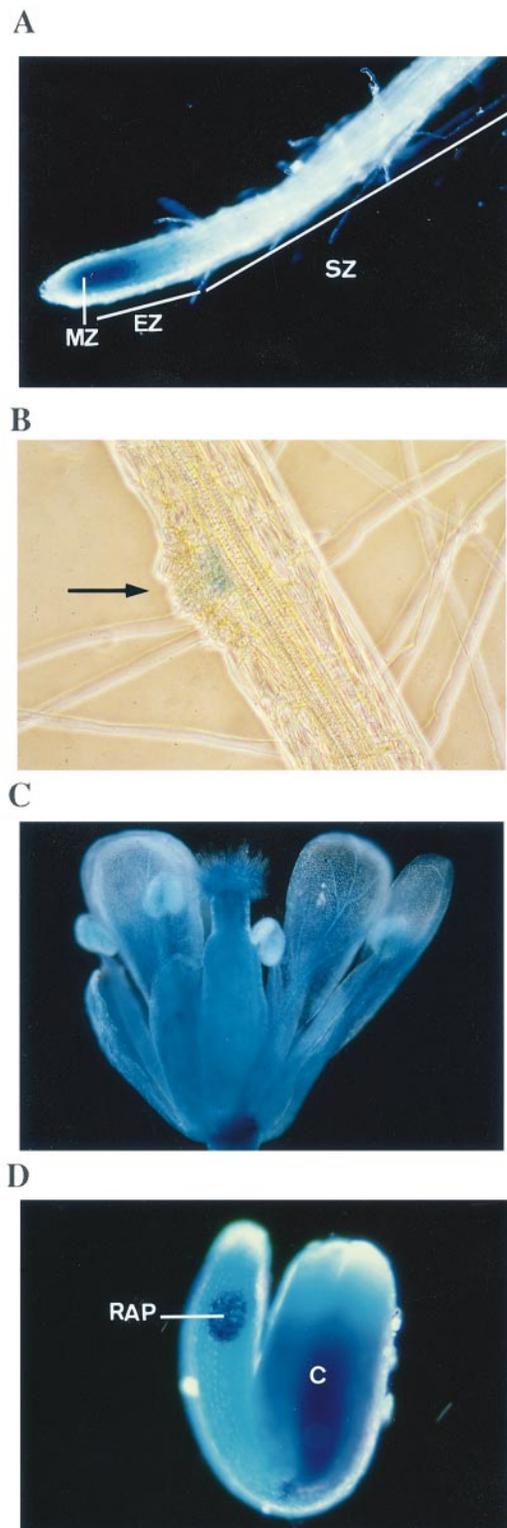


Fig. 2. Histochemical localization of GUS activity in the RPE T-DNA tagged *Arabidopsis* line. (A) Root apex. The meristematic (MZ), elongation (EZ) and specialization zones (SZ) are indicated. (B) Lateral root primordia. (C) Flower. (D) Mature embryo from dry seed. RAP, root apical meristem; C, cotyledon.

detected. The *RPE* cDNA is 1259 nucleotides long with an open reading frame (ORF) of 281 amino acids, and with 5'- and 3'-untranslated regions (UTR) of 106 and 311 (268 + 43) nucleotides, respectively (Figure 4A).

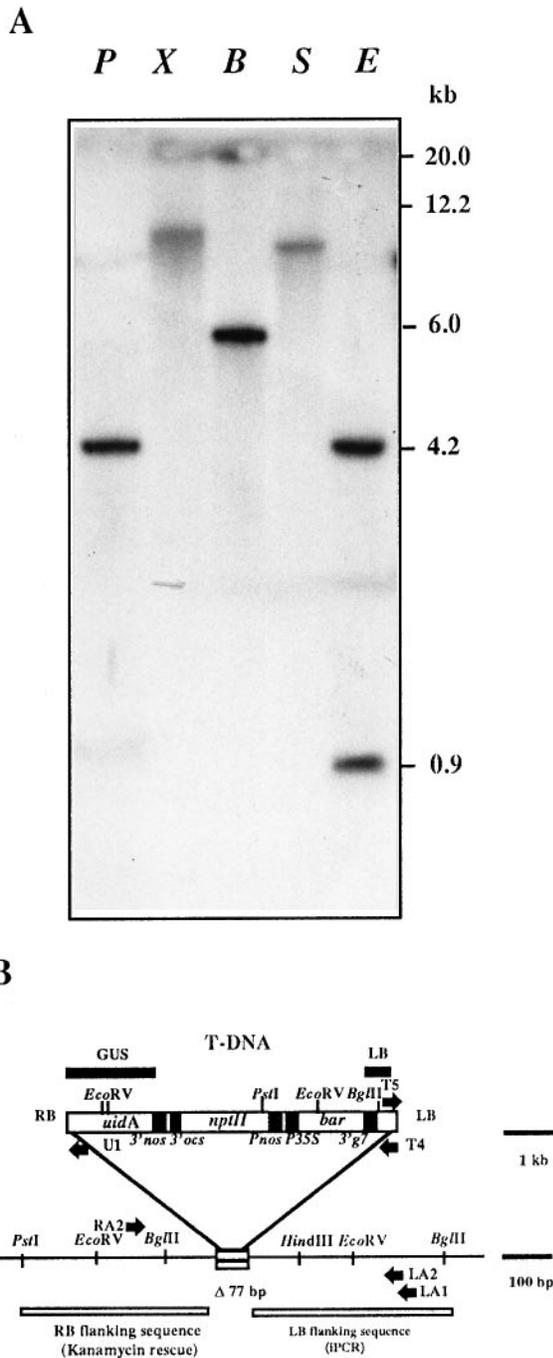


Fig. 3. Molecular analysis of the insertional mutation at the *RPE* locus. **(A)** Southern blot analysis of digested DNA from RPE line carrying a single T-DNA copy. The blot was hybridized with GUS probe. *B*, *Bgl*II; *E*, *EcoRV*; *P*, *Pst*I; *S*, *Sma*I; *X*, *Xba*I. **(B)** Partial restriction map of the *rpe* gene mutated by insertion of the T-DNA. Arrows indicate coding sequences and black boxes indicate promoter and terminal regions. RB and LB correspond to the right and left T-DNA borders, respectively. *uidA*, coding region of the β -glucuronidase (reporter gene) from *E. coli*; *3' nos* and *P nos*, 3' region and promoter regions of the nopaline synthase gene; *3' ocs*, 3' region of the octopine synthase gene; *nptII*, neomycin phosphotransferase II; *P 35S*, promoter region of the Cauliflower Mosaic Virus; *bar*, coding region of the basta resistance gene from *Streptomyces hygroscopicus*; *3' g7*, 3' region of gene 7 from the T-DNA of *pTi15955* (Bouchez *et al.*, 1993). The positions of the probes corresponding to *uidA* (GUS) and Left Border (LB) of T-DNA are indicated by thick bars. The striped box designates the genomic DNA deletion after T-DNA integration. Oligonucleotides T4 and T5 used for IPCR, U1, RA2, LA2 and LA1 are indicated by small arrows.

Translation was assumed to begin at nucleotide 107, the first ATG codon of the open reading frame. The context of this ATG does not match the plant consensus sequence (TAAACAATGGCTA; Joshi, 1987). Nevertheless, the nucleotide 3 bp upstream from the initiation codon is a purine, which is highly conserved in eukaryotic genes (Kozak, 1986). The 3' UTR contains two putative polyadenylation signal sequences, AATAAA and a G/T cluster (GTGTTTTT) at 22 bp and 37 bp, respectively, upstream from the polyadenylation site (Dean *et al.*, 1986). Blast search (Altschul *et al.*, 1990) on the Stanford *A.thaliana* database revealed a strong sequence identity (98.6% at nucleotide level), with eight expressed sequence tags (EST) from the *A.thaliana* ecotype Columbia (Figure 4A). cDNA and genomic sequence alignments demonstrated that the *RPE* gene is interrupted by nine introns, including one in the 3' UTR. The T-DNA was inserted into the third intron, and its resulting integration (deletion of 77 bp and insertion of 5 bp filler sequence) placed the ATG of the *uidA* gene in-frame with the *rpe* gene and allowed a functional translational β -glucuronidase gene fusion. Both introns and exons were characterized; they varied in size from 71 to 286 and from 60 to 268 nucleotides, respectively. The consensus sequences for the intron/exon splice junctions correspond to those reported for plants (White *et al.*, 1992). A Southern blot analysis of the WS genomic DNA was performed using the cloned cDNA as a probe. The gene appears as a single copy per haploid genome of *A.thaliana* (Figure 4B), even at low stringency hybridization and wash conditions (data not shown).

The predicted amino acid sequence of the RPE protein was compared to the NCBI database by the Blast network service. The protein is closely related to RPEs (EC 5.1.3.1) across species, with two conserved regions (Figure 5). RPE catalyzes the reversible interconversion of ribulose-5-phosphate and xylulose-5-phosphate in the pentose phosphate pathway. In particular 85, 81 and 80% of the resulting amino acids were identical to those of chloroplastic RPE from spinach (Nowitzki *et al.*, 1995), potato (Teige *et al.*, 1995) and rice (protein sequence deduced from rice RPE cDNA obtained by RACE PCR with primers localized on rice ESTs D39200 and D48105), respectively. This high degree of conservation between monocotyledonous and dicotyledonous plant sequences is typical for enzymes of sugar phosphate metabolism (Nowitzki *et al.*, 1995). The region where the encoded *Arabidopsis* protein is similar to other bacterial and eukaryotic cytosoles (Figure 5) is preceded by a 46-amino-acid sequence with typical properties of chloroplast transit peptides (Von Heijne *et al.*, 1989; Gavel and Von Heijne, 1990). This region is rich in the hydroxylated amino acids serine (19.6%) and threonine (8.7%). We thus predict that RPE is a plastid-localized protein.

Homologies were found also with the RPE from bacteria and yeast. Amino acid identity was 65% with its homolog from the cyanobacteria *Synechocystis* (Kaneko *et al.*, 1996), and 50 and 48%, respectively, with the corresponding enzymes of *Serratia marcescens* and *Haemophilus influenzae* (Fleischmann *et al.*, 1995). In addition, 42% amino acid identity was found with RPE_YEAST (Miosga and Zimmermann, 1996) and RPE_MYCOB (Figure 5). To determine the relationship between these enzymes, we

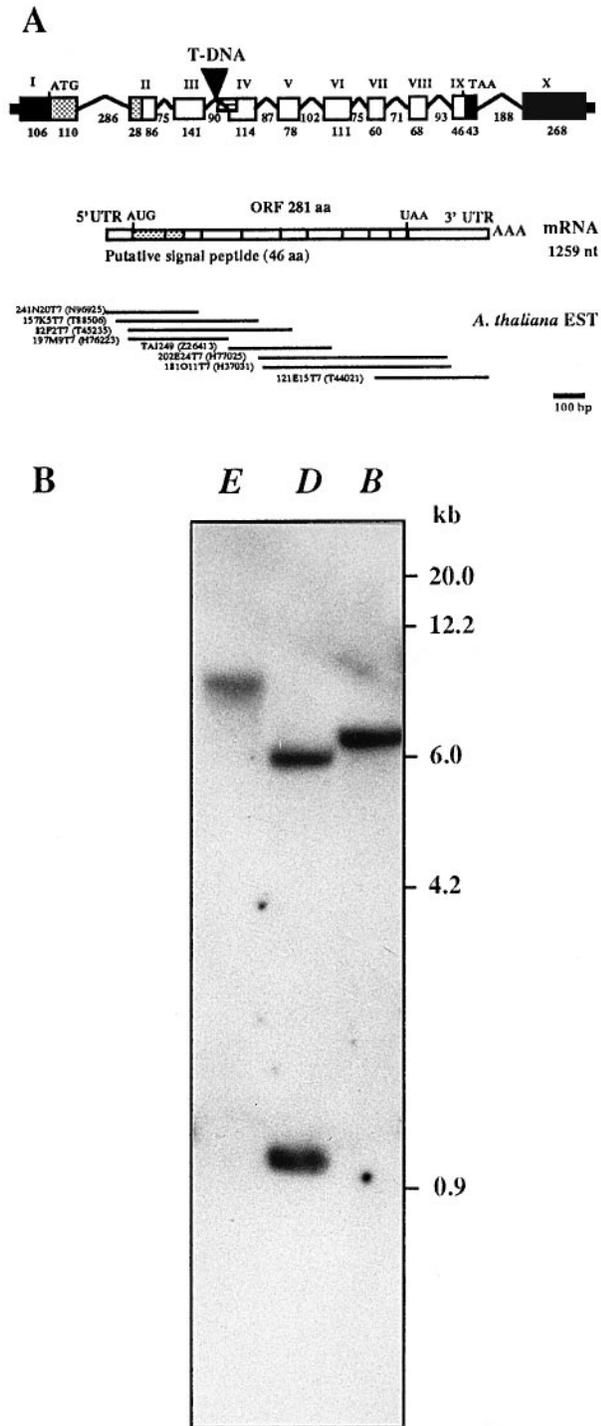


Fig. 4. Organization of the *RPE* gene. (A) Structure of the *RPE* gene and its transcript. Solid black lines designate introns; open boxes designate exons (I–X, determined from cDNA); shaded boxes designate untranslated sequences; the grey box designates putative signal peptide; and the striped box designates genomic DNA deletion after T-DNA integration. The T-DNA is inserted into the third intron. ATG (initiation codon) and TGA (stop codon) are indicated. *Arabidopsis* EST (Expressed Sequence Tagged) showing high homologies with the gene were obtained after a BlastN search on the Stanford *Arabidopsis* database (Altschul *et al.*, 1990). (B) Southern blot analysis of 5 µg genomic DNA isolated from wild-type (WS) *Arabidopsis*. The blot was hybridized with ³²P-labeled *RPE* cDNA. *D*, *Dra*I cut once; while *B*, *Bam*HI, and *E*, *Eco*RI, do not cut in the *RPE* gene.

carried out a phylogenetic analysis with a heuristic tree-building program. Plant *RPE* sequences clustered and formed a lineage distinct from that of yeast *RPE*. They are more similar to the cyanobacterial and eubacterial homologs than to the other eukaryotic sequence, *RPE_YE-AST* (Figure 6).

Finally, the *RPE* gene was mapped on the *A.thaliana* CIC genomic library (Creusot *et al.*, 1995). Four positive YAC clones carrying the *RPE* gene were identified by hybridization. These four clones (CIC3A3, CIC4D8, CIC9G7 and CIC10A10) have previously been anchored to chromosome 5 (contig 29) near the *SEP5A* and the *LFY3* RFLP markers (Schmidt *et al.*, 1997).

Expression of *RPE* genes in plants

To investigate the expression of the *RPE* gene, we isolated total RNAs and poly(A)⁺ RNAs from galls induced by *M.incognita*, and uninfected root tissues (root meristems, lateral root initiation sites and non-meristematic root fragments) of *in vitro*-grown wild-type *Arabidopsis* plants. RNA blots were not sufficiently sensitive to measure *RPE* gene expression because it was difficult to isolate enough tissue and/or because of the low level of *RPE* mRNA expression, as observed in roots (Teige *et al.*, 1995). Since no gene was available as a control in the feeding cell, we used competitive PCR to amplify and quantify cDNA copies from low-abundance *RPE* mRNAs (Gilliland *et al.*, 1990; Kaneko *et al.*, 1992). The target *RPE* cDNA was coamplified with primers RA2 and LA2 in the presence of a dilution series of a competitor DNA of known concentration, which differs from the *RPE* cDNA by a small deletion of 35 bp (Figure 7A). The quantification of *RPE* expression was performed on root tissues from 3 to 15 d.p.i. This time-course experiment showed that the quantity of *RPE* mRNA in galls is constant (data not shown). The results obtained at 15 d.p.i. showed that no *RPE* mRNAs were detected in the non-meristematic root fragments, and very few mRNAs were detected in the lateral root initiation sites. The highest quantity of the *RPE* mRNA was observed in the root meristems and giant cells of NFSs induced by *M.incognita* (Figure 7B and C).

To test the *RPE* upregulation in plant NFSs, we infected potato plants with *Meloidogyne chitwoodi* and the cyst nematode *Globodera rostochiensis*, two very important pathogens on potato crops. Two-week-old galls and syncytia and root tissues from uninfected plants were analyzed for *RPE* mRNA expression. The competitive PCR was carried out with potato-*RPE*-specific primers and a competitor issued from potato cDNA. There was as much potato *RPE* mRNA in the galls as in the root meristems, whereas there was 30% less in the syncytia (Figure 7C). Therefore, the regulation of *RPE* in potato is similar to that found in *Arabidopsis*.

Rescue of *rpe* mutant and complementation

Because the homozygous *rpe* mutant is impaired in sugar phosphate and lethal, we investigated whether exogenous carbohydrate addition would lead to the rescue of the *rpe* mutant. When grown *in vitro* on medium that provides 2% sucrose, kanamycin-resistant dwarf plants appeared with a 1:2 ratio. These plants had light-green leaves and reduced root systems with few lateral roots (Figure 8A). These seedlings developed slowly and died when trans-

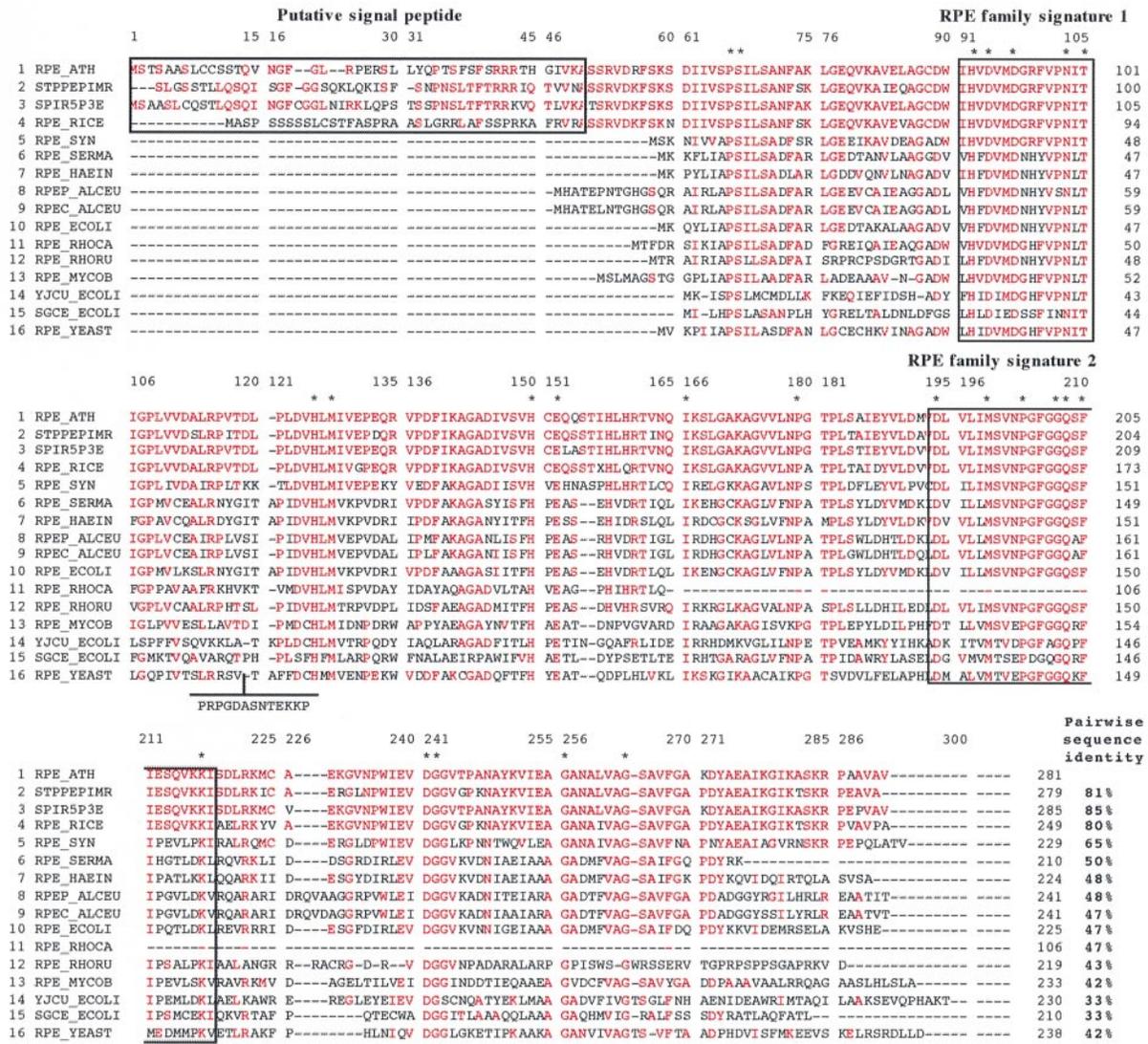


Fig. 5. Sequence alignment of the deduced amino acid sequence of *Arabidopsis* RPE (RPE_ATH) with RPE-related protein sequences. Sources of sequence are *Solanum tuberosum*, STPPEPIMR (Z50098, Teige et al., 1995); *Spinacia oleracea*, SPIR5P3E (L42328, Nowitzki et al., 1995); *Oriza sativa*, RPE_RICE, *Synechocystis* sp., RPE_SYN (D90911, Kaneko et al., 1996); *Serratia marcescens*, RPE_SERMA (P45455); *Haemophilus influenzae*, RPE_HAEIN (P44756, Fleischmann et al., 1995); *Alcaligenes eutrophus*, RPEP_ALCEU (Q04539) and RPEC_ALCEU (P40117, Kusian et al., 1992); *E.coli*, RPE_ECOLI (P32661, Lyngstadaas et al., 1995), YJCU_ECOLI (P32719) and YLHK_ECOLI (P39362, Blattner et al., 1993); *Rhodobacter capsulatus*, RPE_RHOCA (P51012, Larimer et al., 1995); *Rhodospirillum rubrum*, RPE_RHORU (P51013, Falcone and Tabita, 1993); *Mycobacterium tuberculosis*, RPE_MYCOB (Z80108); and *Saccharomyces cerevisiae*, RPE_YEAST (P46969, Miosga and Zimmermann, 1996). Strictly conserved residues are indicated with an asterisk. Identical amino acid residues are colored red. The putative peptide signal and the two conserved regions selected as RPE signature patterns (PDOC00833, Prosite PS01085 and PS01086) are indicated in boxes. The dodecapeptide below the yeast sequence was set apart from the alignment since the corresponding DNA insertion relative to the other sequences is suspiciously intron-like (Nowitzki et al., 1995). The entire sequences of the RPE cDNA from *A.thaliana* and rice are available from DDBJ/EMBL/GenBank under accession Nos AF015274 and AF047444, respectively.

ferred to the soil. We used PCR assay to test for cosegregation of the rescued phenotypes with the *rpe* mutation. PCR experiments were carried out with two RPE primers (LA2 and RA2), which span the *rpe* T-DNA insertion site, and a third primer (U1) specific for the sequence of T-DNA right border (Figure 3C). When genomic DNA from RPE plants was used as a template, both a 437 bp and a 914 bp band were amplified, indicating the presence of both the mutant and wild-type alleles. In contrast, when DNA from dwarf rescue plants was used as a template, only the 437 bp product was obtained from amplifications with all three primers (Figure 8B). This analysis confirmed that all 200 dwarf plants were homozygous for the *rpe* allele.

In an attempt to show genetic complementation of *rpe*, RPE/*rpe* heterozygous plants were transformed by vacuum infiltration with the 35S-CMV::RPE cDNA sense construct. As expected, all of the 50 independent primary transformants selected on hygromycin B and sucrose 2% showed a wild-type phenotype. Of these T1 plants, 11 segregated 100% kanamycin-resistant T2 plants, i.e. homozygous for the *rpe* allele. Transfer of antibiotic-resistant T2 dwarf mutants to agar plates containing hygromycin revealed that all were hygromycin sensitive. Thus these plants did not contain the 35S-CMV::RPE cDNA sense construct. These results confirmed by PCR analysis (data not shown) are in agreement with the complementation of the *rpe/rpe* homozygous mutant by the cloned RPE

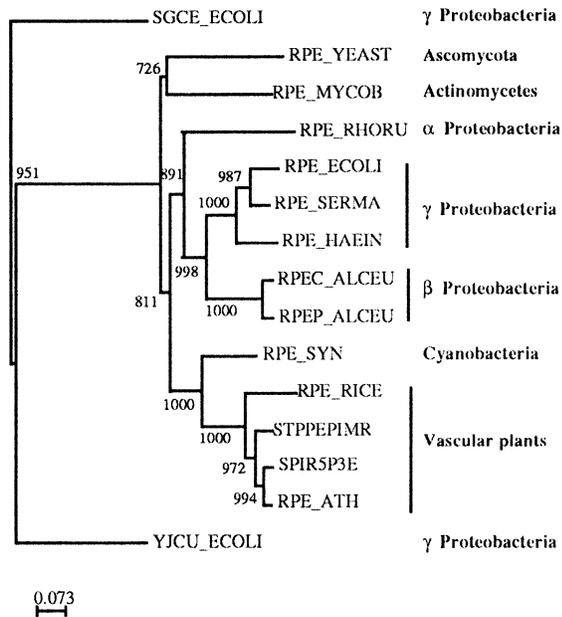


Fig. 6. Unrooted neighbor-joining dendrogram of relationships among plant, yeast and bacterial RPE-related protein. The scale bar represents a genetic distance of 0.073 as the frequency of amino acid substitutions in the pairwise comparison of two sequences according to Kimura's two-parameter method (1980). Bootstrap support (data resampled 1000 times) for the apparent groupings is given. Database sources of sequences are given in the legend of Figure 5.

cDNA and provides additional evidence that the *rpe* phenotype is due to the insertion of the T-DNA into the *RPE* gene.

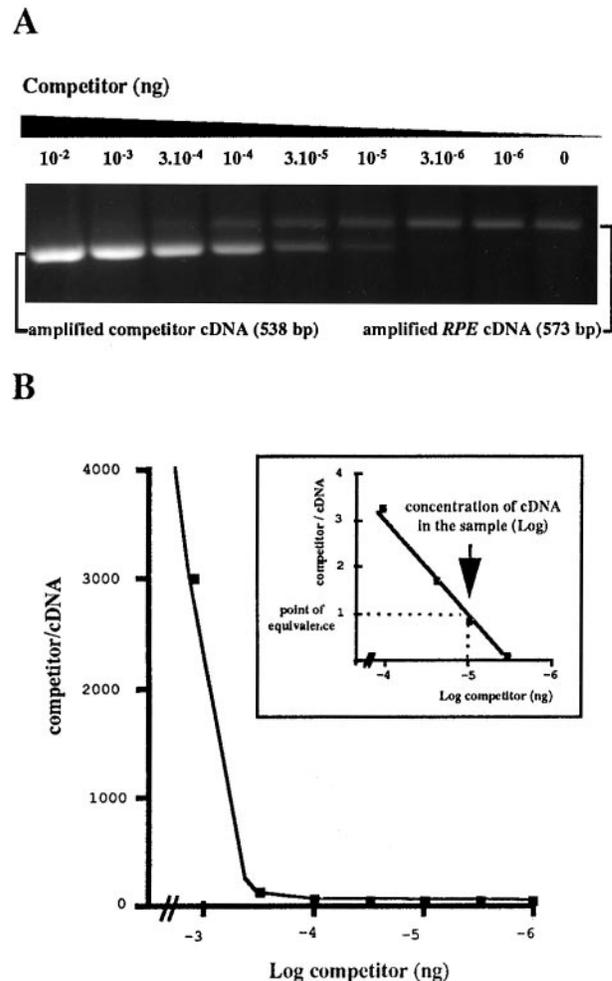
Nematode sensitivity of the rescued mutants

To test the implication of RPE in NFS formation, we have infected the dwarf mutants with *M.incognita* and *H.schachtii*. *In vitro* *H.schachtii* infection of these plants showed that all the plants were attacked with a 2-fold reduction of the number of cysts compared with heterozygous and WS plants tested under the same conditions (Table I). This difference could be explained by the lower number of roots produced by the homozygous plants. However, the number of eggs per cyst and the infectivity of the larvae produced by *rpe* mutants were comparable to those produced by heterozygous and wild-type ones. Conversely, under the same conditions, *in vitro* inoculation of the homozygous plants with *M.incognita* showed that the nematodes are not able to produce galls on most plants (95%). On those rare attacked plants, the number of galls was limited to only one, whereas on heterozygous or control plants, 10–20 galls were generally observed. Moreover, the development of the gall was extremely reduced and later aborted. These results indicate that the morphology of the root dwarf mutant does not affect the behavior of nematodes, and show that, in contrast to syncytia formation, RPE is necessary for giant cell formation.

Discussion

Isolation of an upregulated nematode-responsive gene

We have isolated, by promoter trapping, an upregulated nematode-responsive gene, *RPE*, in *Arabidopsis*. This



C

	<i>A. thaliana</i>	<i>S. tuberosum</i>
Nonmeristematic root fragments	0	0
Lateral root initiation	10 ⁻⁶ ng	3.10 ⁻⁶ ng
Root meristems	10 ⁻⁵ ng	3.10 ⁻⁵ ng
Galls (<i>Meloidogyne</i> spp.)	10 ⁻⁵ ng	3.10 ⁻⁵ ng
Syncytia (<i>G.rostochiensis</i>)	ND	10 ⁻⁵ ng

Fig. 7. Competitive PCR for quantification of *RPE* mRNA in *Arabidopsis* and potato. (A) Target *Arabidopsis* *RPE* cDNA from galls competed against various amounts of competitor-deleted cDNA (10⁻²–10⁻⁶ ng). The primers RA2 and LA2 give a 573 bp fragment when *RPE* cDNA is amplified and a 538 bp fragment when the competitor is amplified. (B) Plot of the ratio of competitive template versus target cDNA after amplification. The inset shows an expansion of the 10⁻⁴–10⁻⁶ range. The point of equivalence (i.e. where there is a 1:1 ratio) represents the concentration of cDNA in the sample (arrow). (C) Quantification of *RPE* transcripts by competitive PCR in *Arabidopsis* and potato. Galls and syncytia were harvested at 15 d.p.i. Root tissues were obtained from uninfected plants.

A



B

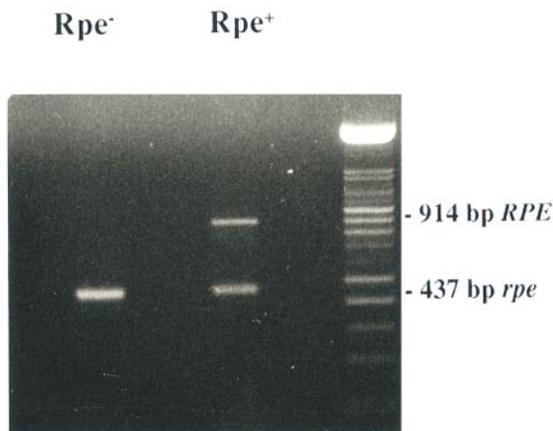


Fig. 8. The *rpe* mutant phenotype. **(A)** The plant on the left is a heterozygous *+/rpe*-tagged line, and the two plants on the right show the homozygous *rpe/rpe* mutant phenotype when grown *in vitro* on selective (kanamycin) medium supplemented with sucrose 2%; this is a dwarf plant with an abnormal root structure and light-green leaves. **(B)** PCR analysis was used to identify seedlings carrying a wild-type *RPE* (914 bp product) and/or mutant T-DNA-tagged *rpe* (437 bp product) alleles. PCRs were performed using two *RPE* primers (RA2 and LA2) and a T-DNA right-border-specific primer, U1.

gene is likely to be directly involved in the biochemical make-up of giant cells. *RPE* gene encodes a key enzyme in the pentose phosphate pathway. The *RPE* gene was cloned after T-DNA tagging by promoterless *gusA* constructs by using *in planta* transformation (Bechtold *et al.*, 1993). This is the first report of the isolation of an upregulated gene in NFSs through a tagging strategy. In comparison to classical *in vitro* transformation (Koncz *et al.*, 1989; Kertbundit *et al.*, 1991; Topping *et al.*, 1991), tagged lines obtained with *in planta* transformation showed lower frequency of GUS activity (7%). Nevertheless, this

Table I. Nematode sensitivity of the rescued *rpe* mutants

	<i>M.incognita</i>	<i>H.schachtii</i>	
	Galls plant ^a	Cysts/plant ^a	Eggs/cyst ^b
Wild type	12.9 ± 3.1	8.2 ± 1.6	301 ± 16
<i>+/rpe</i>	13.0 ± 3.2	7.4 ± 2.4	271 ± 21
<i>rpe/rpe</i>	0.1 (small)	3.8 ± 1.3	320 ± 25

Heterozygous *+/rpe* and the homozygous *rpe/rpe* mutant plants were tested, *in vitro*, in the same plates, on medium supplemented with sucrose 2% and kanamycin. *Arabidopsis* wild-type WS were grown in the same conditions, except that no kanamycin was present.

^aThe number of galls and cysts per plant was determined 4 weeks after inoculation. Values represent the means ± SE obtained on 25 plants, each inoculated with 100 surface-sterilized J2 of *M.incognita* or *H.schachtii*.

^bThe number of eggs per cyst was counted 2 months after infection.

frequency seems to be in better accordance with a random event of T-DNA insertion in the *Arabidopsis* genome in the proximity of a plant promoter. However, this difference could be due to the structure of the vector itself, to specific differences in the transformation and selection process, or in the copy number of T-DNA inserts (Bouchez *et al.*, 1993). The average copy number in the tagged lines obtained is 1.5 copies per transformant. In addition, the occurrence of inverted repeats over the right border (20%) may have reduced the frequency of GUS activation.

Screening for GUS expression after *M.incognita* infection showed that expression of many genes normally expressed at different developmental times or in different cell types are upregulated in giant cells. These results in *Arabidopsis* are consistent with previous observations in tomato (Bird and Wilson, 1994) and confirm the complex morphological and physiological changes in cells during their modification into nematode feeding site (Jones, 1981; reviewed in Atkinson *et al.*, 1994; Niebel *et al.*, 1994).

Roles of RPE in plants

RPE (EC 5.1.3.1) (also known as pentose-5-phosphate 3-epimerase) catalyzes the reversible interconversion of ribulose-5-phosphate and xylulose-5-phosphate. In plants, RPE is integral to both the oxidative pentose phosphate pathway (OPPP) and the reductive Calvin cycle (Dennis and Turpin, 1990; Schnarrenberger *et al.*, 1995). *Arabidopsis* appears to possess only a single, nuclear-encoded, plastid-located RPE enzyme, as in potato (Teige *et al.*, 1995) and spinach (Nowitzki *et al.*, 1995; Schnarrenberger *et al.*, 1995). In this report, we present data on the first *rpe* mutant in plants. The homozygous *rpe* mutant plants fail to germinate without exogenous carbohydrate and can be partially rescued with added sucrose. Another plant mutant, the *chlorina42*, which encodes a light-regulated chloroplast protein of unknown function, has been shown to be rescued on sucrose-containing medium (Koncz *et al.*, 1990). The RPE expression in the photosynthetic tissues and the phenotype of the light-green mutant rescued in a sucrose-containing medium are consistent with the implication of this enzyme in sugar phosphate recycling of the Calvin cycle. Nevertheless, RPE is not expressed only in green tissues containing chloroplasts, as described previously (Teige *et al.*, 1995), but also in specific root cellular types at sites of phloem unloading where

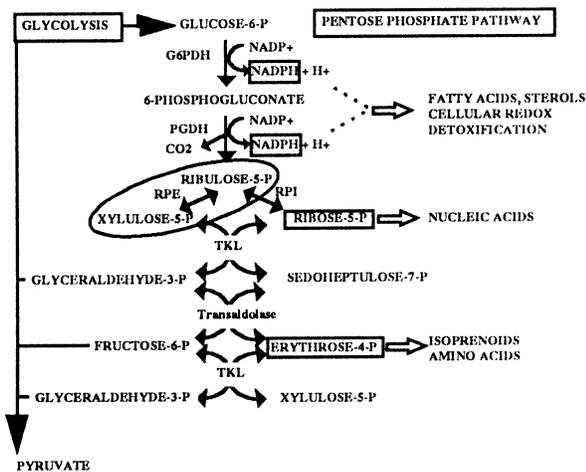


Fig. 9. Glucose metabolism via the oxidative pentose phosphate pathways and relationship with glycolysis. Principal cellular functions of the pathways are indicated, and roles of NADPH included. G6PDH, glucose 6-phosphate dehydrogenase; PGDH, 6-phosphogluconate dehydrogenase; RPE, ribulose-5-phosphate 3-epimerase; RPI, ribose 5-phosphate isomerase; TKL, transketolase.

consumption of carbon skeletons is high. The *RPE* transcript was detected in the proliferating cells of root tips and in the small subset of cells involved in the initiation of lateral roots. Lateral roots are produced when meristems develop *de novo* from one or a few cells within the pericycle, a differentiated tissue surrounding the vascular cylinder (Peterson and Peterson, 1984). In contrast, expression is never observed in the root cap and in the root specialization zones, which cells enter as they acquire their differentiated attributes (Schiefelbein and Benfey, 1991). The pattern of *RPE* expression in roots is very similar, if not identical, to those observed in *Arabidopsis* for other genes implicated in cell division cycle competence or growth, such as mitotic-like cyclin *CYC1At* (Ferreira *et al.*, 1994) and cyclin-dependent kinase *CDC2a* (Hemerly *et al.*, 1993). The *RPE* expression in cotyledons of mature embryos may be correlated to the DNA endoreduplication during cell polyploidization (Brown *et al.*, 1991). These results confirm that a high flux through the OPPP supports a high rate of biosynthesis in actively growing cells. The OPPP plays a crucial role in cells by producing NADPH that is required in numerous biosynthetic reactions (e.g. fatty acids and isoprenoid compounds such as sterols), and by generating carbohydrate intermediates for the synthesis of nucleotides and cell-wall polymers (Figure 9) (Wood, 1986).

The degree of conservation between the *A.thaliana* and spinach sequences suggests that *RPE* also encodes a functional enzyme. Plant *RPE* is more similar to the homologs of cyanobacteria and eubacteria than to the sequence from yeast. The gene phylogeny for these sequences supports the hypothesis that higher plants have obtained their *RPE* gene from eubacteria (perhaps cyanobacteria) via endosymbiotic (organelle to nucleus) gene transfer (Nowitzki *et al.*, 1995), similar to the scenario observed for chloroplast GADPH (Martin *et al.*, 1993) and chloroplast 3-phosphoglycerate kinase (Brinkmann and Martin, 1996).

RPE roles in feeding cells

We showed that the *RPE* gene is affected during nematode parasitism and is necessary for the giant cell formation. *RPE* is upregulated in the early steps of feeding cell formation induced by *Meloidogyne* spp. but not by cyst nematodes (*H.schachtii* and *G.rostochiensis*). This is consistent with the admitted hypothesis that giant cells and syncytia, despite analogous ultrastructure, differ completely in the way they are formed (Jones, 1981). Giant cells develop by repeated mitosis without cytokinesis and subsequent cell expansion. The multinucleate state of syncytia results from cell-wall dissolution and coalescence of adjacent cells. In this case, cell division and metaphase chromosomes have never been reported. However, in *A.thaliana*, key regulators of the cell cycle, *CDC2a* and *CYC1At*, are induced early in the initial steps of both NFS formation (Niebel *et al.*, 1996). The lack of early *RPE* expression in syncytia agrees well with previous ³H-thymidine incorporation experiments which indicate that syncytia are relatively quiescent in terms of DNA synthesis, in contrast to giant cells, which undergo heavy synthesis in early steps of induction (Gheysen *et al.*, 1997).

Later, *RPE* accumulates in both types of developing NFSs throughout the nematode life cycle. This expression in both NFSs (15 d.p.i.) is in accordance with the establishment of an active metabolic sink that provides food to the adult females, which require a considerable amount of nutrients for egg production.

From a general point of view, it has been shown that the pentose phosphate pathway plays a crucial role in the host–protozoa parasite relationship. It maintains a pool of NADPH, which serves to protect against oxidant stress and generates carbohydrate intermediates used in nucleotide and other biosynthetic pathways (Barrett, 1997). The human parasite *Plasmodium falciparum* also stimulates host cell activity (Atamna *et al.*, 1994), and deficiency in the first enzyme of the pathway, glucose-6-phosphate dehydrogenase, protects human erythrocytes from infection (Ruwende *et al.*, 1995). In giant cell formation, pronounced activities of glucose-6-phosphate and 6-phosphogluconate dehydrogenases found in histochemical preparations (Endo and Veech, 1969) together with the activation of *RPE* seem to be in accordance with the role of the OPPP in host–parasite interaction.

The implication of *RPE* in root meristems and initiation sites of lateral roots indicates that the genetic control of NFS and the first stages of meristem formation share common steps. This result confirms that root cells undergo metabolic reprogramming when they turn into NFSs. Some animal parasites, such as *Trichinella spiralis*, initiate similar changes in differentiated mammalian muscle cells (Jasmer, 1995). These results support the hypothesis that ‘normal’ biochemical functions have been recruited to play key roles in halting pathogen growth (Opperman *et al.*, 1994b). Determining the mechanisms underlying *RPE* expression at the onset of feeding-cell formation should give insight into the molecular basis of this morphogenetic event.

Towards engineering plant nematode resistance

The mechanisms by which nematodes influence plant cell metabolism should share regulatory features in different plant species, since a range of sedentary nematode species

are able to develop NFSs in numerous host plants (Goddijn *et al.*, 1993). We demonstrated that the *RPE* gene in potato is regulated in a manner similar to that in *Arabidopsis* after infections by sedentary parasitic nematodes. This result confirms that *Arabidopsis*, besides being ideally suited for genetic dissection of development (Meyerowitz, 1989), is also a good host plant model for studying molecular interactions with parasitic nematodes (Sijmons *et al.*, 1991; Niebel *et al.*, 1994). The identification and cloning of nematode-responsive plant genes and promoters constitute a major challenge for our knowledge and understanding of the plant–nematode interaction and the development of novel approaches to engineering plant resistance (reviewed in Gheysen *et al.*, 1996; Grundler, 1996). Nematode-responsive promoters may be used to express anti-nematode proteins (Atkinson *et al.*, 1994), phytotoxic proteins or essential genes (anti-sense approaches) that interfere with the development of feeding cells (Sijmons, 1993). Furthermore, determining how a nematode selects particular root cells and modifies them to serve as a feeding site will enhance our understanding of normal cell development and may serve in identifying genes that regulate aspects of cell division.

Materials and methods

Plant materials and infection with nematodes

A T-DNA mutagenized *A.thaliana* (L.) Heyn seed collection (3000 lines) was obtained and characterized previously in INRA Versailles (Bechtold *et al.*, 1993). The plant transformation vector pGKB5, designed for promoter trapping and gene tagging (Bouchez *et al.*, 1993), was used to transform wild-type *A.thaliana*, ecotype Wassilewskija (WS) by vacuum infiltration. Plants were grown in a greenhouse on sand at 20°C under a light/dark regime of long days (16 h light and 8 h darkness). The seedlings were incubated at 4°C for 2 days in the dark for cold treatment. T-DNA tagged T3 seedlings were selected with Basta herbicide. Three-week-old lines were inoculated with 100 *M.incognita* second-stage juveniles (J2). One and 2 weeks after inoculation, the plants were carefully harvested by rinsing in water and were GUS-stained as described below. For *in vitro* analyses of seedlings, seeds were surface-sterilized and grown on Gamborg B5 medium (Sigma) containing 1% sucrose, 0.8% agar (plant cell culture tested, Sigma) and 50 µg/ml kanamycin. Kanamycin resistance was scored in 2-week-old seedlings. For *in vitro* nematode infection, 100 surface-sterilized freshly hatched J2 of *M.incognita* or *H.schachtii* were added on each 2-week-old seedling as described previously (Sijmons *et al.*, 1991). The plates were kept at 20°C in a cycle of 16 h light/8 h darkness. GUS induction time course experiments were done daily after nematode infection. Potato plants *Solanum tuberosum* L. cv Désirée were grown in a greenhouse and inoculated with *M.chitwoodi* J2 or *G.rostochiensis* cysts under the same conditions.

Histochemical localization of GUS activity

β-glucuronidase (GUS) activity was assayed histochemically with X-Glu according to Jefferson *et al.* (1987), modified by the use of buffer 0.5 mM K₃Fe(CN)₆ and 0.5 mM K₄Fe(CN)₆ in 0.1 M NaH₂PO₄ pH 7, to prevent potential staining artefacts (De Block and Debrouwer, 1992). For microscopic analyses, galls were excised from X-Glu-stained plants, fixed for 30 s in a microwave oven (temperature 50°C fixed) in freshly prepared 2% formaldehyde/4% glutaraldehyde in 50 mM sodium phosphate buffer pH 7.2 (PBS), washed with PBS, embedded in OCT Compound (BDH Laboratory Supplies) and frozen in nitrogen-cooled isopropane. Frozen embedded material was sectioned at 6 µm (at –20°C) using a Leica JUNG CM 1800 cryomicrotome. Whole-mount *Arabidopsis* and cryosections were observed through a Zeiss Axioplan 2 microscope with dark-field illumination to increase the sensitivity of the detection.

Southern blot and RNA analysis

For Southern blot analysis, genomic DNA was isolated from 4-week-old plants grown *in vitro* as described by Doyle and Doyle (1990). Southern blots were performed as described in Sambrook *et al.* (1989),

using 5 µg of genomic DNA. For *RPE* RNA analysis on wild-type *Arabidopsis* and potato, galls and syncytia were excised after nematode infection. For comparison, root tissues (non-meristematic root fragments, root tips, initiation sites of lateral roots) were obtained from uninfested plants. All tissues were harvested rapidly and with a minimum of manipulation, immediately frozen in liquid nitrogen and stored at –80°C until use. Total RNA was isolated as described by Chomczynski and Sacchi (1987). Poly(A)⁺ RNA was isolated with Quickprep Micro mRNA Purification Kit (Pharmacia P-L Biochemicals Inc.) according to the manufacturer's instructions. RNA samples were separated in a formaldehyde-containing agarose gel, blotted and hybridized as described in Sambrook *et al.* (1989).

Isolation of T-DNA flanking sequences

Kanamycin plasmid rescue of T-DNA right-border flanking sequence was performed as described by Bouchez *et al.* (1996). The genomic DNA from transgenic plants was digested by *Pst*I (unique site at the beginning of the *nptII* gene) and ligated into pResc38 vector. *Escherichia coli* DH12S cells (Gibco-BRL) were transformed with the ligation mixture. Ampicillin and kanamycin selection provided clones containing the complementing region from the T-DNA plus a flanking genomic DNA region. IPCR amplification of the T-DNA left-border flanking sequences was performed as described by Earp *et al.* (1990). Genomic DNA of transgenic plants was digested by *Bgl*III and ligated with T4 DNA ligase. The PCR was done with T-DNA oligonucleotides T4 (5'-GTA-CAT-TGC-CGT-AGA-TGA-AAG-ACT-3') and T5 (5'-CTA-CAA-ATT-GCC-TTT-TCT-TAT-CGA-C-3') as depicted in Figure 3C. The oligonucleotides T4 and T5 hybridize 500 and 300 bp away from the 3' T-DNA left border, respectively. Amplification was performed with a cycle of 1 min denaturation at 94°C, 1 min annealing at 58°C and 2 min polymerase extension at 72°C, repeated 35 times, with a final 10 min 72°C extension in an MJ Research PTC-200 Peltier Thermal Cycler. The amplified fragment was cloned and sequenced with T7 polymerase sequence kit (Pharmacia).

RACE (Rapid Amplification of cDNA Ends)

The 5' end of the *RPE* cDNA was cloned using the 5' RACE kit (Gibco-BRL) with poly(A)⁺ RNA from the wild-type *A.thaliana* ecotype WS (Frohman *et al.*, 1988). First-strand cDNAs were made using the *RPE* antisense-specific primer LA1 (5'-CCT-TGA-ACG-CAT-TCG-CTG-GAG-TGA-C-3'). After C-tailing, PCR amplification was performed using an anchor primer (5'-CTA-CTA-CTA-CTA-CTA-GGC-CAC-GCG-TCG-ACT-AGT-ACG-GGI-IGG-GII-GG G-IIG-3') and a nested antisense *RPE*-specific primer LA2 (5'-GAT-ACC-TTC-TCT-GCA-CAC-ATT-TTC-C-3'). The PCR cycle was 94°C for 1 min; 55°C for 2 min; 72°C for 2 min; repeated 35 times, with a final 10 min 72°C extension. The 3' end of the *RPE* cDNA was cloned using first-strand cDNAs made with a primer oligo(dT) (5'-TGA-AGG-TTC-CAT-GAA-TCG-ATA-GGA-ATT-CTT-TTT-TTT-TTT-TTT-TTVN-3'). Each PCR was done with the primer 3RACE (5'-TGA-AGG-TTC-CAT-GAA-TCG-ATA-G-3') and the specific primer RA2 (5'-CAG-AAG-GAG-AAC-TCA-TGG-AAT-TG-3'). PCR products were cloned in pUAG (Ingenium) and sequenced.

Mapping

The *RPE* gene was mapped on the *A.thaliana* (ecotype Columbia) CEPH-INRA-CNRS (CIC) yeast artificial chromosome (YAC) genomic library (Creusot *et al.*, 1995). Membrane hybridization screening was performed using the *RPE* cDNA as a probe.

Competitive RT-PCR for quantification of mRNA

Poly(A)⁺ RNAs from galls induced by *M.incognita* and WS *Arabidopsis* root tissues were reverse transcribed using the First-Strand cDNA Synthesis Kit (Pharmacia) with the oligo(dT) primer. The resulting first strand cDNAs were used as template for PCR amplification. To quantify accurately the *RPE* cDNA abundance, a competitor matrix was created by deleting 35 bp (*Eco*RV–*Stu*I fragment) from the RA2-LA2 PCR-amplified *RPE* cDNA (573 bp). A precise dilution series was prepared, ranging from 1 to 10^{–7} ng of competitor template (538 bp). We performed an initial PCR titration against a broad range of dilutions in log increments. We then performed a second finer titration over a narrower range of dilutions for precise quantification. The accuracy of this method is improved through the use of master mixes. We prepared a master mix containing, per reaction, oligonucleotides RA2 and LA2 10 pmol each, dNTP 200 µM each, 1× PCR buffer, *taq* DNA polymerase 0.5 U (Appligene) and 1 µl of first-strand cDNA. We added 90 µl of this mixture to 10 µl of previously prepared competitive template. PCRs

were performed according to the following temperature profile (35 cycles): 94°C, 1 min; 55°C, 1 min; 72°C, 2 min. Since any change in the variables that influence the efficiency of the PCR will affect equally the yield of both competitive and target cDNA PCRs, the relative ratio of the two products remains unchanged. The relative amounts of each PCR product were readily quantified by direct scanning with a densitometer of ethidium-stained 2% agarose gels. The amount of competitor is multiplied by the ratio of target cDNA bp per competitor bp to correct for increased ethidium staining per mole of the larger fragment. The competitor product per target product was plotted as a function of the starting concentration of competitor. At the point where cDNA and competitor products are in equivalence, the starting concentration of RPE cDNA added to the PCR is equal to the known concentration of the competing deleted cDNA.

Competitive RT-PCRs were performed on poly(A)⁺ RNA from galls induced by *M.chitwoodi*, syncytia induced by *G.rostochiensis* and potato root tissues. The primers RP2 (5'-CTT-TCA-CCA-GGA-GGA-GAA-TTC-A-3') and LP2 (5'-CTC-AAG-TCC-GAG-ATT-TTC-TT-3') were complementary, respectively, to the 5'- and 3'-ends of the *S.tuberosum* RPE-coding sequence. In this case, the competitive template is a 50-bp-deleted potato RPE cDNA obtained after *MseI* restriction.

Plant transformation

For the sense expression of the RPE cDNA in transgenic plants of *A.thaliana*, the complete RPE cDNA was amplified on *A.thaliana* (WS) cDNA using 5' (5'-GTC-CCG-GGT-CAA-CCT-CCG-CCG-CTT-CCT-TG-3') and 3' primers (5'-GTC-CCG-GGG-ACA-AAT-GAA-ATG-CTT-TAT-TAT-AAG-TC-3'). The *SmaI* fragment was ligated into the binary vector pCAMBIA 1302 digested by *NcoI* and *BstEII*. The proper orientation with regards to the 35S-CMV promoter was verified by sequencing. The sense construct was introduced into *A.tumefaciens* strain C58C1 and used to transform heterozygous *Rpe/rpe A.thaliana* plants by vacuum infiltration (Bechtold *et al.*, 1993). Primary transformants were selected on GB5 medium containing 2% (w/v) sucrose and 75 µg/ml hygromycin B and transferred to soil. Plants were grown to maturity and allowed to self.

DNA sequence analysis

The BLAST search program (Altschul *et al.*, 1990) was used for sequence analysis and comparisons in the DDBJ/EMBL/GenBank and swissProt databases at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/>) and in the *Arabidopsis* Database (AtDB) Project (<http://genome-www.stanford.edu/Arabidopsis/>). Multiple sequence alignments and the unrooted neighbor-joining dendrogram were determined using CLUSTAL W (Thompson *et al.*, 1994). The significance of the phylogenetic results was assessed using bootstrap analysis (Felsenstein *et al.*, 1985). PROSITE (<http://expasy.hcuge.ch/sprot/scnpsit1.html>) allows a protein sequence to be scanned for signature patterns.

Accession numbers

The sequence data of the *A.thaliana* and rice RPE cDNA have been submitted to the DDBJ/EMBL/GenBank databases under accession Nos AF015274 and AF047444, respectively.

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