Ranbpm homologue genes characterised in the cyst nematodes Globodera pallida and Globodera 'mexicana' ☆

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Abstract

A homologue of a Ran binding protein to microtubules (*ranbpm*) gene, initially known as IC5, was identified and characterised in second-stage juveniles (J2s) of the potato cyst nematode *Globodera pallida*. The full-length cDNA (937 bp) was obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE) and specific primers were designed to amplify the genomic sequences of 2396 bp containing six introns. The ORF (798 bp) encodes a putative 265 amino acid sequence with a predicted SPRY domain and a signal peptide of 23 amino acids on the N-terminal part of the protein. In situ hybridisation experiments showed that the transcript is located in the dorsal gland of the J2s, suggesting that the encoded protein has an extracellular function and can be involved in the late stages of parasitism such as feeding site establishment. This gene was specifically over-expressed in the juveniles before and during parasitism, but not in adult developmental stages. As this gene is presumed to be involved in plant–nematode interaction, particularly in the development and maintenance of the feeding structure that allows the nematode to achieve parasitic development, homologous genes were sought in other cyst nematode species. One of them was cloned and sequenced in the closely related species *Globodera* 'mexicana'.

1. Introduction

Potato cyst nematodes are responsible for severe damage in cultivated Solanaceous plants throughout the world. This damage represents 300 million dollars in potato crop yield in Europe every year [1]. Plant resistance is an effective and environmentally friendly way of controlling potato cyst nematodes. However, its application to *Globodera pallida* is hampered by the few sources of resistance yet to be identified [2]. In order to circumvent this problem, artificial resistances can be developed [3] but require good knowledge of the genes implicated in plant–nematode interaction.

These quarantine nematodes develop an intimate relationship with their host via the induction of a complex structure, a feeding site otherwise known as the syncytium, in the vascular cylinder of the potato roots. This syncytium is induced 48 h p.i. and used by the cyst nematode to withdraw nutrients from its host. The three oesophageal glands of the cyst nematodes are presumed to be involved in parasitism, because they are the production site of the secretory products that can be injected into plant cells via the nematode stylet [4]. Secretions from the two subventral glands are often involved in the penetration and migration steps, while the dorsal gland secretions seem to be involved in feeding site formation [4,5]. Indeed, the development and functioning of the syncytium are among the most complex responses induced by a nematode in plant tissues. The cyst nematodes choose one cell and then induce cell wall collapse in adjacent cells. Syncytium induction involves both proliferation of endoplasmic reticulum, ribosomes, mitochondria and plastids in the cell, and enlargement of the nucleus without mitosis, a phenomenon known as endoreduplication [6].

Thus, secretory products are preferential targets for studying plant–nematode interactions due to their key role in parasitism. Several genes corresponding to such secretory products have

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already been identified in cyst nematodes. Many correspond to proteins with cellulolytic activity such as cellulase [7–9], pectate lyase [10] and expansin [11] and are involved in the migratory process. Few correspond to proteins putatively involved in the development and maintenance of the feeding site, one example being chorismate mutase [12].

Among the genus *Globodera*, *Globodera pallida* and *Globodera* 'mexicana' are genetically close species that can be differentiated through their host range [13,14]. *G. pallida* develops on *Solanum tuberosum* whereas *G. 'mexicana'* develops on *Solanum nigrum*, but not on *S. tuberosum* [14]. In a previous study, the transcriptome of these two species was compared by SSH [15]. One of the SSH fragments, designated as GPLIC5, was identified after subtraction of *G. pallida* transcripts by *G. 'mexicana'* transcripts and was found to match the A18 factor. This A18 factor was isolated in *Globodera rostochiensis* by cDNA-AFLP and had homology with Ran binding protein to microtubules (RanBPM) genes [16].

The purpose of this study was to gain new insight into the IC5 gene. In situ hybridisation was carried out to localise the transcript and its full-length sequence was isolated by RACE-PCR. Data presented in this paper sheds new light on the expression pattern and secretion of the protein derived from this gene that appears to encode a RanBPM. Subsequently, a homologue was cloned in *G. 'mexicana'* and potential functional differences were sought by comparing *G. pallida* and *G. 'mexicana' ranbpm* genes.

2. Materials and methods

2.1. Nematodes

G. pallida population Guiclan (France) was mainly used in this study. The cysts were obtained from potato crops (*S. tuberosum* var. Désirée) in laboratory conditions. In order to stimulate juvenile hatching (J2s), the cysts were soaked in water for 4 days and then in potato root diffusate at room temperature. The J2s were stored in water at 4 °C for in situ hybridisation, or at −80 °C after centrifugation for nucleic acid extraction.

2.2. Isolation of 5′ and 3′ cDNA ends

2.2.1. RNA isolation

Total RNA was extracted from 50 to 100 mg of J2 with the RNeasy Mini kit (Sigma) according to the manufacturer's instructions. The RNAs were treated with DNase I (10 U, Ambion) for 15 min at 37 °C and then purified by phenol/chloroform/isoamylic alcohol (25/24/1) extraction. The RNAs were precipitated with sodium acetate 3 M and absolute ethanol, and stored at −80 °C.

2.2.2. mRNA reverse transcription

Reverse transcription was performed in a mix containing 2 µg of total RNA, 0.25 µM of d(T)$_{25}$ primer and 8.5 µl of RNase-free water. The samples were heated for 10 min at 70 °C and set on ice. 1X superscript buffer, 2.5 mM MgCl$_2$,

0.25 µM of each dNTP and 0.01 µM of DTT were then added. The sample was heated for 1 min at 42 °C. Superscript III (Invitrogen, 200 U/µl) was added and the mix was incubated at 42 °C for 50 min, and at 70 °C for 15 min. One microlitre of RNase mix (Boehringer) was added and the mixture was incubated for 30 min at 37 °C.

2.2.3. 5′ and 3′ RACE

5′ and 3′ RACE [17] were performed using the 3′ and 5′ RACE System for rapid amplification of cDNA ends (invitrogen) according to the manufacturer's instructions. The 3′ end of the transcript was amplified by PCR using two microlitres of the first strand cDNA, 1X buffer, 1.5 mM of MgCl$_2$, 0.2 mM of each dNTP, 0.4 µM of AUAP primer (Table 1), 0.4 µM of an internal specific primer of the SSH fragment (GPLIC5 Fwd; Table 1) and 1 U of Taq polymerase (Promega). PCR was performed with 30 cycles at 96 °C for 20 s, 58 °C for 20 s and 72 °C for 30 s, with a final elongation step at 72 °C for 5 min.

5′ RACE reaction: the first strand cDNA was obtained from total RNAs using 0.4 µM of specific primer GPLIC5Rev2 (Table 1). Nested PCR was performed in 50 µl with 1X buffer, 1.5 mM of MgCl$_2$, 0.2 mM of each dNTP, 2.5 units of Taq DNA polymerase, 5 µl of cDNA, 0.4 µM of AAP, and 0.4 µM of the internal specific primer (GPLIC5Rev2 or GPLIC5Rev3; Table 1). PCR conditions were 35 cycles at 96 °C for 20 s, 53 °C for 30 s and 72 °C for 30 s, with a final elongation step at 72 °C for 5 min.

The PCR products were purified (GeneElute PCR Clean-Up kit, Sigma) and cloned in pGEM-T Easy Vector System I (Promega) according to the manufacturer's instructions.

2.3. Cellular localisation and developmental expression

2.3.1. mRNA in situ hybridisation

The GPLIC5Fwd and GPLIC5Rev2 primers (Table 1), designed from the GPLIC5 SSH clone, were used to amplify the insert and to synthesise digoxigenin (DIG)-labelled sense and anti-sense probes by asymmetric PCR amplification.

Insert amplification was performed in 50 µl with 30 ng of insert, 0.4 µM of each primer, 1X buffer, 0.2 mM of each

Table 1
The primers used in this study are symmarised in the table

Name	Sequences (5′→3′)
RACE primers	
GPLIC5fwd	GGCTATTTTTGGGGGTCAC
GPLIC5Rev2	CGTATGAGTAAGTGCCT
GPLICFRev3	TCACTTCATAGTAGAAAATGCC
AUAP	GGCCACGCGTCGACTAGTAC
AAP	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG
Other primers	
5′IC5	TTTTTATTGCCCCCAAAATG
5′IC5.2	AAATGCGCACCTTTCTCTTC
3′IC5.3	GCAAACCCATCATAAATTCTCG
3′IC5.2	CATGATCAATAGGCAAACCG
GPFARF	TCGCTGCCGCCCATTGAT
3′GPFAR	GGCGCCCTCAGTCTTCAGCA

dNTP, 2 U of Taq DNA Polymerase (Promega) and 1.5 mM MgCl$_2$, for 4 min at 94 °C, 40 cycles at 94 °C for 30 s, 56 °C for 30 s, 72 for 1 min, and a final elongation step of 4 min at 72 °C. Asymmetric PCR was performed in 40 µl with 2 µl of the purified insert, 2.5 µM of a primer (Fwd or Rev), 1X buffer, 1.5 mM of MgCl$_2$, 0.75X Dig-dUTP/dNTP mix (Roche Applied Science) and 2 U of Taq DNA polymerase (Promega) for 4 min at 94 °C, 34 cycles at 94 °C for 30 s, 55 °C for 1 min, 72 °C for 90 s, and a final elongation step of 4 min at 72 °C. In situ hybridisation was performed as described by De Boer et al. [18] with slight differences. Freshly hatched J2s were fixed in fixation buffer (including paraformaldehyde) for 18 h at 4 °C and 4 h at room temperature. The nematodes were cut with a razor blade and the sections were permeabilised with proteinase K for 30 min at 22 °C before freezing at -80 °C. Hybridisations were performed at 45 °C with purified sense or anti-sense single strand cDNA probes. The signal was detected using alkaline phosphatase immunostaining (NBT-BCIP, Boehringer).

2.3.2. ranbpm gene expression analysis

One to five nematodes were used to extract the RNAs. They were crushed with a modified Pasteur pipette, and recovered in a mix containing RNase-free water, 1 µl of d(T)$_{25}$ primer at 10 µM and 1 µl of RNasin. The mix containing the crushed nematodes was incubated for 10 min at 70 °C and then a solution containing 1X buffer, 2.5 µM MgCl$_2$, 0.4 µM of each dNTP and 0.01M of DTT was added and incubation was resumed for 1 min at 42 °C before adding 200 U of Superscript III (invitrogen). The reaction was incubated for 50 min at 42 °C, and then for 15 min at 70 °C. DNase I was used as previously described in RNA isolation.

The IC5 cDNA was amplified using 5 µl of the reverse-transcribed mRNAs in a mix containing 1X buffer, 1.5 mM of MgCl$_2$, 0.2 mM of each dNTP, 0.4 µM of each specific primer (5′ IC5.2 and 3′ IC5.2; Table 1) and 2.5 units of Taq DNA Polymerase (Promega). As a positive control, the Gp-far−1 gene reference was amplified in the same conditions with the specific primers GPFARF and 3′ GPFAR (Table 1). PCR conditions were 5 min at 96 °C, 35 cycles at 96 °C for 20 s, 58 °C for 30 s, 72 °C for 30 s with an additional second per cycle, and a final elongation step for 5 min at 72 °C.

2.4. Identification of the genomic sequence

2.4.1. DNA isolation

Genomic DNA was extracted from the eggs of 500 rehydrated cysts. The frozen eggs were crushed with a piston. The DNA was extracted with lysis buffer (0.1 M Tris, pH 8, 10 mM EDTA, 2% SDS) and proteinase K, and then incubated at 65 °C for 1 h. The DNA was precipitated and purified with 5M NaCl and 10% CTAB, incubated for 10 min at 65 °C and then with a phenol/chloroform (1:1) mix. The DNA was precipitated with ammonium acetate 5 M overnight at 4 °C. A mix containing 30% of PEG 6000 and isopropanol (99.8%) was added to achieve DNA precipitation. The sample was treated with 1 µl of RNase A (500 µg/mL) for 2 h at 37 °C.

2.4.2. ranbpm gene amplification

The 5′ IC5.2 and 3′ IC5.3 primers (Table 1) designed from the IC5 cDNA sequence were used to amplify the *ranbpm* gene from 100 ng of genomic DNA. The PCR products were checked on 1% agarose gel and purified using GenElute PCR product kit (Sigma). The purified PCR products were cloned in the pCR2.1 vector using the TA cloning kit (invitrogen) according to the manufacturer's instructions. The minipreps were performed using the GenElute Plasmid Miniprep kit (Sigma) according to the manufacturer's instructions.

2.5. Sequence analysis

The cloned inserts were sequenced by Macrogen (Korea) or using our own facilities (ABI310 machine, Perkin). WU-Blast2 Parasite Genomes Database Query on EMBL-EBI (www.ebi.ac.uk/blast2/parasites.html), and Blast on NCBI (www.ncbi.nlm.nih.gov/) or on nemaBLAST (http://www.nematode.net/) were used to search for sequence homologies. The Expasy translate tool (http://us.expasy.org/tools/dna.html) was used to predict amino acid sequences. Sequence alignments were performed with Multalin algorithm [19] (http://prodes.toulouse.inra.fr/multalin/multalin.html). The sequence signatures were detected using SignalP algorithm [20] (www.cbs.dtu.dk/services/SignalP/), and ELM program [21] (http://elm.eu.org/). The Radar program [22] (http://www.ebi.ac.uk/Radar/) was used to identify repeats in amino acid sequences.

3. Results

3.1. Isolation of the full-length ranbpm cDNA in G. pallida and sequence analysis

Specific RACE-PCR primers were designed from the GPLIC5 SSH fragment. The GPLIC5Fwd primer was used to amplify the 3′ end of the GPLIC5 transcript. A single band of approximate size 500 bp was visualised. GPLIC5Rev2 was used to amplify the 5′ end of the GPLIC5 transcript but did not produce the entire cDNA. Another primer, GPLIC5Rev3, was therefore used to obtain the entire 5′ end sequence. Once again, a single band of 500 bp was visualised on agarose gel after electrophoresis of the PCR products. These PCR products were cloned and sequenced. Using overlapping regions, a putative full-length cDNA of 937 bp was built in silico (Fig. 1). An ORF of 798 bp was identified and two specific primers (5′ IC5 and 3′ IC5.3) flanking this ORF were designed to check the in silico construction. The product obtained was about 800 bp, in keeping with the expected size of 824 bp. The corresponding sequence perfectly matched the IC5 transcript sequence built in silico and encoded a predicted protein of 265 amino acid with a predicted molecular weight of 29 kD. Marked similarities (E-value: 7.10^{-43}, of 2 817 606 sequences available in Genbank) to the A18 factor from *G. rostochiensis* [16] and *ranbpm* genes from different mammalian, insect or fungal species (E-value $< 10^{-9}$, of 2 817 606 sequences available in Genbank) were obtained using BlastP. Similar results were obtained with the WU-Blast2 or Nemabase databases that
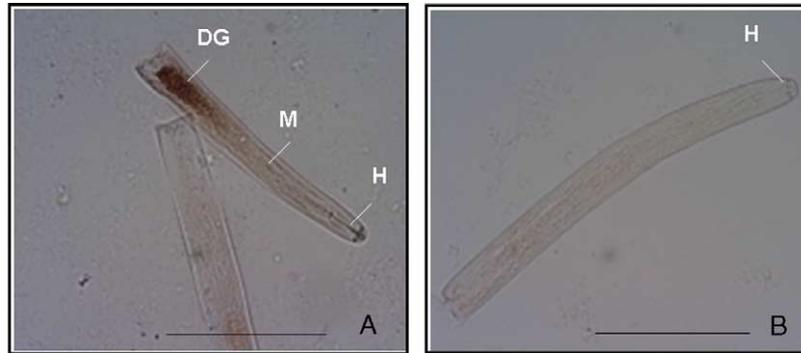
Fig. 1. Nucleic acid and amino acid sequence representation of *Globodera pallida ranbpm* cDNA. The start and stop codons are identified in the nucleotidic sequence by two boxes. The putative peptide signal of 23 amino acids is underlined on the 5′ side of the transcript, and the SPRY domain is identified by a grey box. The tandem repeated sequence is shown in bold characters with arrows indicating each beginning of the repetition.

confirm the sturdiness of the blasts. SignalP 3.0 predicted the presence of a signal peptide of 23 amino acid in the N-terminal end of the protein (Fig. 1) indicating that the product of this gene is most probably secreted. The Radar program identified a tandem repeated sequence of 25 amino acids downstream of the start codon (Fig. 1). A motif search in the ELM database identified a SH3 ligand domain ('KPNKKVKG') in this region. A SPRY domain (domain in SPla and the RYanodine receptor) was identified beginning at position 121 and ending at position 249 (E-value: 3.9E-20). Although of unknown function, this domain was identified in different proteins particularly, in association with LisH motifs that are involved in the regulation of microtubule dynamics.

3.2. In situ localisation of G. pallida ranbpm transcripts

The tissue localisation of the *ranbpm* transcript was analysed in *G. pallida* J2s by in situ hybridisation. The anti-sense digoxigenin-labelled DNA probe clearly showed a specific accumulation of *ranbpm* transcripts in the dorsal gland of the infective juveniles (Fig. 2(A)). No signal was observed using the GPLIC5fwd probe as a negative control (Fig. 2(B)).

3.3. Expression pattern of the G. pallida ranbpm gene

The developmental expression pattern of the *ranbpm* gene was characterised by RT-PCR amplification of part of

Fig. 2. Localisation by in situ hybridisation of the *rbp*-1 transcript in the dorsal oesophageal gland of *Globodera pallida* infective juveniles. J2s were hybridised by anti-sense GPLIC5Rev2: (A) and sense GPLIC5Fwd; (B) digoxigenin-labelled DNA probes. H, head; M, metacorpus; DG, dorsal gland; Bar, 70 µm

the corresponding transcripts in five developmental stages of *G. pallida* (eggs, hatched J2s, J2s after penetration into roots, males, and females). RNAs from each stage were extracted and reverse transcribed and the cDNAs were amplified with specific primers (5′ IC5.2 and 3′ IC5.2). Gene expression was detected in eggs, pre-parasitic J2s and hatched J2s for which the strongest band was visualised at about 700 bp (Fig. 3(A)). No such IC5 gene expression was detected in the male and female stages whereas the constitutive GPFAR-1 cDNA fragment (492 bp) used as a positive control was amplified (Fig. 3(B)) in all the developmental stages.

3.4. Characterisation of the ranbpm gene in G. pallida and other Heteroderidae species

Genomic DNA of three *Globodera*, four *Heterodera* and one *Meloidogyne* species was used to amplify the genomic ORF of the *ranbpm* gene. Amplification products were obtained only with the *G. pallida* and *G. 'mexicana'* DNA. A band of about 2500 bp (Fig. 4) was obtained, suggesting the presence of introns in the *ranbpm* (*rbp*) gene with an ORF of only 798 bp. A second band was observed in *G. 'mexicana'* at about 2400 bp, suggesting that the *rbp* gene belongs to a multigenic family.

The two genes thereafter designated as *Gp-rbp*-1 and *Gm-rbp*-1 were cloned and sequenced. Their sizes were, respectively, 2396 and 2544 bp. The intron/exon boundaries were determined by aligning *Gp-rbp*-1 and *Gm-rbp*-1 with the corresponding *G. pallida* cDNA sequence. Six introns were identified in the two species at the same positions. Intron number 3 was located between the two 25 amino acid repeated motifs identified (Fig. 1). Intron size ranged from 42 to 890 bp (Fig. 5) and was relatively conserved between *G. pallida* and *G. 'mexicana'* except for intron number 2 which was 100 bp smaller in *Gp-rbp*-1 than in *Gm-rbp*-1. The sequences of the corresponding introns were highly conserved, ranging from 95.2% for intron number 2 to 100% identity for intron number 4. *Gp-rbp*-1 and *Gm-rbp*-1 showed only two indels of three nucleotides (one in *Gp-rbp*-1 and one in *Gm-rbp*-1) and one non-synonymous substitution resulting in 98.9% similarity between the coding regions of these two genes. Amino acid alignment of *G. pallida*, *G. 'mexicana'* and the closest *ranbpm* homologue found in the databases (AJ251757) for *G. rostochiensis* is shown in Fig. 6. Only 43.7% sequence similarity was observed between *Gp-rbp*-1 and the *G. rostochiensis* RanBPM derived protein. Focusing on the signal peptide and SPRY domains, the comparison of the two sequences showed, respectively, 52% (18 aa) and 58% (128 aa)
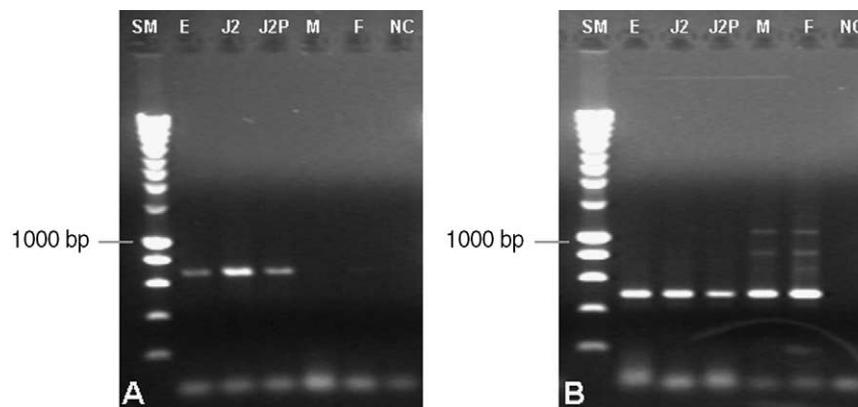

Fig. 3. Expression pattern of the *rbp*-1 transcripts in different developmental stages of *Globodera pallida*:(A) PCR products obtained with *rbp*-1 specific primers (5′ IC5.2 and 3′ IC5.2); (B) PCR products obtained as a control with the constitutively expressed gene *Gp-far*-1 (specific primers: GPFARF and 3′ GPFAR). E, eggs; J2, hatched juveniles; J2P, juveniles after penetration into roots; M, males; F, females; NC, negative control; SL, Smart ladder (Eurogentec).
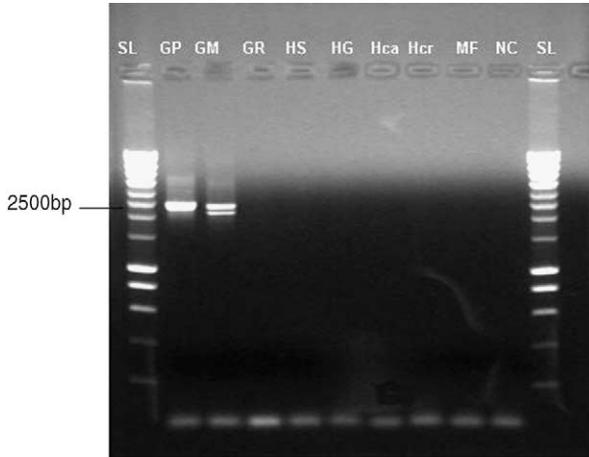
Fig. 4. PCR amplification of the *rbp-1* gene in different species of *Globodera, Heterodera,* and *Meloidogyne*. GP, *Globodera pallida*; GM, *G. "mexicana"*; GR, *G. rostochiensis*; HS, *Heterodera schachtii*; HG, *H. glycines;* Hca, *H. carotae;* H. cr, *H. cruciferae;* MF, *Meloidogyne fallax;* NC, negative control; SL, smart ladder (Eurogentec). The DNAs were from INRA collection except the *H. glycines* DNA which was provided by K. Lambert.

sequence identity, indicating that both domains tend to be more conserved compared with the rest of the sequence.

4. Discussion

Few genes with a potential role in the late events of parasitism, such as establishment of the syncytium, have been identified. Although *ranbpm* homologues sequences found in cyst nematodes exist in GenBank databases, this is the first study of the isolation and characterisation of *ranbpm* genes in *G. pallida* (*Gp-rbp*-1) and *G.* 'mexicana' (*Gm-rbp*-1). Candidate genes for pathogenicity are expected to correspond to products that can be secreted by the nematode. The predicted proteins from *Gp-rbp*-1 and *Gm-rbp*-1 both had a small size (MW: 29 kDa) compatible with the exclusion size of the lumen

of the nematode stylet. Protein secretion was supported by the identification of a signal peptide motif on the N-terminal side, as usually found in other secreted proteins [23]. Localisation of the *Gp-rbp*-1 transcripts in the dorsal oesophageal gland cell of the J2s was obtained by in situ hybridisation. As this oesophageal gland is activated after the subventral ones [24], we can assume that the *Gp-rbp*-1 gene is more likely involved in the latest stages of parasitism, such as feeding site development and maintenance. Other genes such as chorismate mutase or the *Skp*1 genes [12,23] display similar transcript localisation in accordance with their putative role in the sedentary stages of parasitism. All these results provide indirect evidence of RanBPM protein secretion in the host plant by the nematode. It will be of considerable importance to develop of antisera capable of recognising this RanBPM protein to be able to confirm that it is secreted in planta and identify its plant cellular target.

In *G. pallida*, the *Gp-rbp*-1 gene is expressed in the egg and J2 stages, but never detected in adult stages. This expression pattern has already been observed with other genes implicated in plant–nematode interactions such as the pectate lyases identified in *M. incognita* [25], or one copy of chorismate mutase identified in *M. incognita* and in *G. pallida* [26,27]. However, our data differ slightly from those published by Qin et al. [16] who indicated that the expression pattern of the *G. rostochiensis* A18 factor is J2-stage specific. Expression of the *Gp-rbp*-1 gene obtained in the eggs is surprising but cannot be explained by genomic DNA amplification because the amplification product would have been larger due to the presence of introns. However, as a few J2s can also be found in non-stimulated eggs, this can lead to misinterpretation of the egg expression pattern. Further investigations will be needed to determine *Gp-rbp*-1 gene expression pattern more precisely in quantitative terms.

During parasitism, cyst nematodes induce a feeding site which is characterised by genome multiplication with polytene
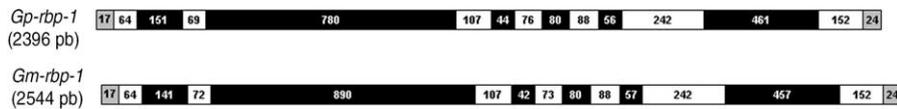

Fig. 5. Organisation of the *rbp*−1 genes isolated in *Globodera pallida* and *G.* 'mexicana' species, *Gp-rbp*-1 and *Gm-rbp*−1. Introns (black boxes), exons (open boxes) and non-transcribed sequences (grey boxes) are represented with the corresponding lengths (in bp) indicated in each box.
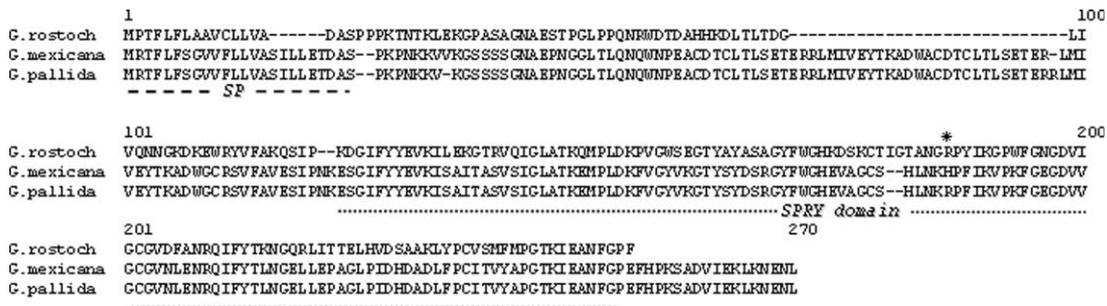

Fig. 6. Alignment of the predicted RBP-1 proteins in *Globodera pallida*, *G.* 'mexicana' and *G. rostochiensis* (G. rostoch) (AJ251757). Conserved amino acids are in grey boxes, the signal peptide is dash underlined and the SPRY domain is dot underlined. The unique substitution observed between the *G. pallida* and *G.* 'mexicana' proteins is indicated by an asterisk.

nuclei where chromocentre size enlarges due to the endor-eduplication mechanism [28]. Several sequence data converge to strongly support a specific role of the RBP-1 protein secreted by *G. pallida* and *G.* 'mexicana' in the development and maintenance of the feeding site as a key step of the parasitic cycle. Firstly, RanBPM-like proteins are ubiquitous and can be found independently of parasitic interactions. This suggests that the proteins RanBPM (RBP-1) normally associates with are available in plants. Secondly, weak similarities were identified between *rbp*-1 and RanBPM-like proteins of the free-living organisms and particularly, in the nematode *C. elegans* (E-value: 10^{-8}), whereas strong similarities (E-value: 10^{-42}) were identified with RanBPM of other cyst nematode species. Furthermore, a SPRY domain was identified in an RBP-1 predicted protein. In some cases, this domain appears to correspond to a RYyanodine receptor with an RNA binding role [29]. In other cases, the SPRY domain is associated with a LisH motif involved in the regulation of microtubule dynamics. No LisH domain was identified in predicted RBP-1, but a domain linked to a SH3 protein with a potential role in cytoskeleton organisation has been identified in both *Gp-rbp*-1 and *Gm-rbp*-1. Clearly, functional investigations are now needed to assess the exact role of *rbp*-1. In this way, the RNAi method will be of interest, if accurate observations of the syncytium can be achieved in order to affirm that the phenotype observed is well induced by the inactivated gene.

Among the Heteroderidae family different species exist and have different host ranges. Consequently, it is worthwhile to study the variability of the *rbp*-1 gene in different species. The primers used allowed us to detect the gene only in *G. pallida* and the closely related species, *G.* 'mexicana'. The high conservation of the protein sequence observed between *Gp-rbp*-1 and *Gm-rbp*-1 (98.9%) and their common intron distribution strongly suggest that they are orthologous copies. Six introns of 42–890 bp were observed in *rbp*-1 genes. In the opposite, a high degree of variability was observed between *G. rostochiensis* and *G. pallida* protein sequences. Compared to the cellulase genes, the similarity observed (56.3%) for *ranbpm* genes is quite low as 93% similarity was found between *G. pallida* and *G. rostochiensis* cellulases [15], and 71.4% between *G. rostochiensis* (GR-eng1) and *Heterodera glycines* (HG-eng2) cellulases [30]. Therefore, the *ranbpm* isolated *in G. rostochiensis* could well be a paralogous copy of *rbp*-1. *Ranbpm* genes identified in *G. pallida* and *G. rostochiensis* may have different roles in plant–nematode interaction as was shown by Gao et al. [23,31] for cellulases HG-ENG5 and HG-ENG6 which have only 42–47% similarity and different enzymatic properties.

No amplification was observed with the other nematode species tested despite the fact that RanBPM-like ESTs were already sequenced in *G. rostochiensis* and *H. glycines*. Assuming that *rbp*-1 is involved in feeding site initiation, there is no reason why this gene should be absent from *Globodera* and *Heterodera* species that all display the same syncytium phenotype regardless of the host plant. In order to obtain homologues, alignment of sequences and designing primers to conserved regions or an alternative approach such as library screening would be required. However, the presence of *rbp*-1 in *Meloidogyne* remains questionable as root-knot nematodes induce morphologically similar feeding sites (giant cells) but using different mechanisms. For example, in root-knot nematodes, feeding site development is characterised by an increase in the number of nuclei through polyploidisation mitosis that involves mitosis without cell division but not endoreduplication like in cyst nematodes [28]. We can assume that if *ranbpm*-like genes exist in *Meloidogyne*, they probably have a different role in plant parasitic interaction and may also be quite divergent in sequence compared to *rbp*−1. Further investigations are underway to find *ranbpm*-like genes in *Heterodera* and *Meloidogyne* species. This will give new insights into the specificity of *rbp*-1 in plant–nematode interaction.

Acknowledgements

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