ACCELERATED PUBLICATION

Induction of ASABF (*Ascaris suum* antibacterial factor)-type antimicrobial peptides by bacterial injection: novel members of ASABF in the nematode *Ascaris suum*

Ajitha PILLAI¹, Satoshi UENO¹, Hong ZHANG and Yusuke KATO²

Department of Developmental Biology, National Institute of Agrobiological Sciences, Oowashi 1–2, Tsukuba, Ibaraki 305-8634, Japan

Recently, invertebrate models have been widely used for the study of innate immunity. Nematodes are novel potential candidates because of the experimental advantages of *Caenorhabditis elegans*. However, whether nematodes have active immune responses is still ambiguous. Previously, we reported ASABF (*Ascaris suum* antibacterial factor)-type antimicrobial peptides in the parasitic nematode *Ascaris suum* and the genetic model nematode *C. elegans*. Further screening of a cDNA library and an expressed-sequence-tag database search detected five novel members of ASABF (ASABF- β , - γ , - δ , - ϵ and - ζ) in *A. suum*.

The transcripts for ASABF- α , - β , - γ , and - δ clearly increased in the body wall, and also in the intestine for ASABF- δ , 4 h after injection of heat-killed bacteria into the pseudocoelom (body cavity), suggesting that these peptides are inducible in the acute phase of immune response. These results also suggest that the nematodes can recognize bacteria in the pseudocoelomic fluid and evoke an active immune response.

Key words: defensin, innate immunity, nematode, parasite, Toll.

INTRODUCTION

Antigen-specific adaptive immunity is found only in higher vertebrates. By contrast, innate immunity against non-specific targets, e.g. production of antimicrobial peptides, is observed in a wide range of animals. Thus some invertebrate models are thought to be useful for studying innate immunity, e.g. insects [1] and ascidians [2]. Nematodes, including the genetic model *Caenorhabditis elegans*, seem to be potential candidates because of their experimental utility. To achieve this goal, it is a prerequisite to confirm that nematodes can recognize non-selves, especially microbes, and actively remove them.

Concerning the immune defence activity directly attacking microbes, we previously reported microbicidal activity, lysozyme-like bacteriolytic activity and agglutinating activity in the pseudocoelomic (body-cavity) fluid in the intestinal parasitic nematode *Ascaris suum* [3]. The microbicidal substance was identified to be an antimicrobial peptide and designated ASABF (*Ascaris suum* antibacterial factor) [4,5], and its homologues were identified in *C. elegans* [6,7]. In addition, homologues of another antimicrobial peptide, amoebapore, were also detected in *C. elegans* [8]. Furthermore, a lysozyme-like gene, *lys-1*, has been reported to be involved in resistance to *Serratia marcescens* [9].

Recently, some specific responses induced by contact with pathogens were reported for *C. elegans*. A bacterial pathogen, *Microbacterium nematophilum*, induces a morphological change in the post-anal region [10]. Contact with another bacterial pathogen, *Serratia marcescens*, induced some specific gene expression, including lysozyme- and lectin-like genes [9].

However, these responses were observed only in contact with pathogens and not with non-pathogenic microbes, i.e. *C. elegans* is cultured on a lawn of a non-pathogenic bacterium, *Escherichia coli* OP50, in laboratories, but such responses have not been detected. Thus it is still ambiguous whether these pathogen-induced responses are typical innate immune responses to non-specific targets or are specific pathological changes caused by these pathogens.

To test whether nematodes can recognize and respond to microbes in the pseudocoelom (body cavity), injection of heat-killed non-pathogenic bacteria can be used as the immunization usually used in insects [11]. Although *C. elegans* is an excellent genetic model organism, it is difficult to collect a sufficient number of bacteria-injected individuals and quantify tissue-specific gene expression because of its small size. In contrast, *A. suum* is big enough (the adult female is 25–35 cm in length) and is suitable for these experiments, so we previously suggested the advantage of combinational analysis using *C. elegans* and *A. suum* [6]. In the present study we identified novel members of ASABF-type antimicrobial peptides in *A. suum*, and tested their inducibility by injection of heat-killed bacteria into the pseudocoelom.

EXPERIMENTAL

PCR

PCR primers used in the present study are listed in Table 1. In the following, '(A, B)' means a PCR reaction using primers A and B. '(A, B) $\times$ (C, D)' means a nested-PCR using primers A and B for

Abbreviations used: ASABF, *Ascaris suum* antibacterial factor; RACE, rapid amplification of cDNA ends; UTR, untranslated region; EST, expressed sequence tag; RT, reverse transcription; EF1 α , elongation factor 1 α ; cybS, the small subunit of cytochrome *b*₅₅₈; SL1, nematode splice leader sequence 1; MAP kinase, mitogen-activated protein kinase.

¹ These authors contributed equally to this work.

² To whom correspondence should be addressed (e-mail kato@affrc.go.jp).

The nucleotide sequences reported in this paper have been submitted to the EMBL, DDBJ, GenBank® and GSDB Nucleotide Sequence Databases and the EBI (European Bioinformatics Institute) Data Bank under the accession numbers D87123, D87124, D87125, AB086058, AB091202, AB029812, AB029813, AB029814, AB029815, AB090347, AB090348, AB090349, and AB090350.

Table 1 List of PCR primers

Name of primer	Sequence
1a-5'f	5'-GATATTGACGAAAAAGACAACTACTGTGCGACC-3'
1a-1as	5'-TTGGCATAGGTACGCTT-3'
1a-2s	5'-CTGGTCATTTTGCCTCAAC-3'
1a-2as	5'-CACATCGATCGCAAAACACAC-3'
1b-5'f	5'-ATAGTTCTGTTGACTTGTGGGG-3'
1b-1as	5'-CTCCTTTTGGCATGGGTACA-3'
1b-2s	5'-ATTGCTGCTGTGGTTGCTGT-3'
1b-2as	5'-CGACCGCATACACAAGTCGGC-3'
1b-s-18V-26C	5'-GTTGCACAGGGTTATGC-3'
1b-as-84G-89R	5'-GCGTCCTTTTTCCTCCT-3'
1c-5'f	5'-TTTGGAGAGAGAGTTCTCCTCACTGCTAACG-3'
1c-1as	5'-GTCAATAGCGTTTACTTTCC-3'
1c-2s	5'-GTGTTATTGTTGCTGTGGTG-3'
1c-2as	5'-CTACCACACCGACTACACACG-3'
1c-s-13P-18V	5'-CCTGGTCTTAGCAAGTC-3'
1c-as-78S-+ 4	5'-GCGTTTACTTTCTACGCTC-3'
1d-5'f	5'-GTTGGCTTATACTGTTTG-3'
1d-1as	5'-GTCAGAGTTCAATTACGTC-3'
1d-2s	5'-CGTATTGATAGTGGTGTGGTTTG-3'
1d-2as	5'-GACTGCATACACAGGTCGGA-3'
anti 1d + 24-2	5'-CAAGCATTTGTTTATGTCAGAG-3'
n1-1s	5'-ATCGTTCTTCTCATGCTCGTCATC-3'
n1-1as	5'-TTTCGATCAGATGGTCATTGTAATTG-3'
n1-2s	5'-CATCTTGTTCGCTTCCACCGATGC-3'
n1-2as	5'-CGGTGATCTTTTCGATCTGCA-3'
n1-3s	5'-GCAITCTTTCTGCAAGTACAGGGA-3'
n2-1s	5'-ACTGACCACTTTTACGGTCGTTAAT-3'
n2-1as	5'-GAAAACACCAGCCTCAGCGTTTAC-3'
n2-2s	5'-GTTAATGGGGGTAGTCCTCAC-3'
n2-2as	5'-GCAAGTTTGACCAAGTATCGAG-3'
n2-3s	5'-GCATCACTTCGTGCAAAATACAGAAC-3'
n2-4as	5'-GCTAAGAAGTGGAGTATCCATACGTG-3'
n2-1s-r	5'-ATTAACGACCGTAAAGTGGTCAGT-3'
n2-1as-r	5'-TAAACGCTGAGGCTGGTGTTC-3'
As-EF1a-1s	5'-CCCTTAGACTGCCTCTCCAAGAC-3'
As-EF1a-1as	5'-CGACCGACTTCACCTCGGTGGTA-3'
As-28S-1s	5'-GTGATCGCATGTTAACGGGTGTG-3'
As-28S-1as	5'-CGCGTCCGCACTTTTGTGCAAT-3'
sense 17	5'-GGATATTGTGAAAAGCGCGG-3'
cb558-sR	5'-GCCGGAGCAACGTCGGCAGCAG-3'
cb558-2as	5'-CTGGAATGAGTGGTACCATCGCA-3'
SL1	5'-GGTTTAATTACCAAGTTTGAG-3'
M4	5'-GTTTCCCGAGTCACGAC-3'

the first round of PCR and primers C and D for the second round of PCR.

cDNA cloning

cDNAs for ASABF- β , - γ and - δ were cloned using a reverse-transcription (RT)-PCR-based procedure. Previously, a cDNA for ASABF- α was cloned using a three-step RT-PCR [4], i.e. amplification of internal regions using degenerate primers (step 1), 5'-rapid amplification of cDNA ends (RACE) using nematode splice leader sequence SL1 primer (step 2) and 3'-RACE (step 3). The PCR products obtained at each step were subcloned into a pGEM-T vector (Promega). The partial sequence of cDNAs for ASABF- β and - γ were found by further screening of the subclones derived from step 2. Full-length cDNAs were cloned by 3'-RACE using the 5'-untranslated (UTR) sequences as specific primers, i.e. (1b-5'f, M4) for ASABF- β , and (1c-5'f, M4) for ASABF- γ . In addition, the partial sequence of a cDNA for ASABF- δ was identified from subclones derived from step 1. The entire sequence of a cDNA for ASABF- δ was cloned by 3'-RACE

and 5'-RACE, i.e. (sense 17, M4) and (SL1, anti 1d + 24-2). A full-length cDNA for ASABF- δ was cloned by 3'-RACE using the 5'-UTR sequence as a specific primer, i.e. (1d-5'f, M4), and its sequence was confirmed. *Taq* DNA polymerase was added during the first denaturation, i.e. a hot-start mode, in all RT-PCR reactions used for the cloning of cDNAs for ASABF- β , - γ and - δ .

On the other hand, the partial sequence of ASABF- ε , and - ζ were first identified in the parasitic nematode expressed-sequence-tag (EST) database (see below). The internal sequences were amplified using specific primers designed using those EST sequences, i.e. (n1-1s, n1-1as) $\times$ (n1-2s, n1-2as) for ASABF- ε , and (n2-1s, n2-1as) $\times$ (n2-2s, n2-2as) for ASABF- ζ . The end of cDNAs were determined by 5'-RACE and 3'-RACE, i.e. (SL1, n1-1as) $\times$ (SL1, n1-2as) and (n1-1s, M4) $\times$ (n1-2s, M4) for ASABF- ε , and (SL1, n2-4as) $\times$ (SL1, n2-1s-r) and (n2-3s, M4) $\times$ (n2-1as-r, M4) for ASABF- ζ .

Identification of exon-intron junction of ASABF genes

The genomic DNA of *A. suum* was prepared as previously described [6]. The introns for ASABF genes were identified by PCR using the genomic DNA as templates, i.e. (1b-s-18V-26C, 1b-as-84G-89R) for ASABF- β , (1c-s-13P-18V, 1c-as-78S + 4) for ASABF- γ , (sense 17, anti 1d + 24-2) for ASABF- δ , (n1-1s, n1-1as) $\times$ (n1-2s, n1-2as) for ASABF- ε and (n2-1s, n2-1as) $\times$ (n2-2s, n2-2as) for ASABF- ζ .

Computer-assisted sequence analysis

Alignment and phylogenetic analysis were carried out using ClustalW (<http://www.ddbj.nig.ac.jp/E-mail/clustalw-j.html>) with compensation by eye-fitting. Secretory signal regions were predicted by the SignalP program (<http://www.cbs.dtu.dk/services/SignalP/>). The theoretical pI was estimated by ExPASy program (http://www.expasy.org/tools/pi_tool.html). Blast database searches were performed to identify novel members of ASABF in the EST database of *A. suum* (<http://www.nematode.net/Species/Summaries/Ascaris.suum/>).

Culture and immune challenge of nematodes

Adult female *A. suum* were obtained from Tokyo Shibaura Zohki, Tokyo, Japan. Before the experiments, the worms were incubated in Eagle's essential medium (Gibco BRL) containing 5 μ g/ml of enrofloxacin at 37 °C for 2 days. An overnight culture of *E. coli* OP50 was autoclaved after washing and resuspended in sterilized saline. The heat-killed bacteria were injected into the pseudocoelom (5×10^7 cells/worm). The injected worms were incubated at 37 °C for 4 h. The body walls, intestines, uterus and ovaries were separately collected from each individual after the incubation.

Quantitative RT-PCR

Each tissue was frozen in liquid nitrogen and ground using a Coolmil (Tokken, Chiba, Japan). Total RNA was isolated using Isogen (Nippon Gene, Toyama, Japan). Single-strand cDNA was synthesized using PowerScript Reverse Transcriptase (Clontech) with random hexamer primers. To estimate the relative accumulation of ASABF transcripts, quantitative real-time PCR was performed using an Applied Biosystems 7700 instrument with SYBR Green. SYBR Green PCR Master Mix (Applied Biosystems) was mixed with 1 μ l of the cDNA solution and specific primers (final concn. 0.2 μ M) in 25 μ l of reaction

Figure 1 Alignment of ASABF-type antimicrobial peptides in *A. suum*

The amino acid sequences for a total of six members are aligned. The secretory signal/mature region junction of ASABF- α [4] and - δ (Y. Kato, unpublished work) was experimentally determined, and that of other members was predicted by the SignalP program. Asterisks (*) indicate conserved cysteine residues. The pro-region of ASABF- α at the C-terminus [4] is represented by dots. The conserved dibasic site near the C-terminus is indicated by '+ '.

mixture, i.e. (1a-2s, 1a-2as) for ASABF- α , (1b-2s, 1b-2as) for ASABF- β , (1c-2s, 1c-2as) for ASABF- γ , (1d-2s, 1d-2as) for ASABF- δ , (n1-2s, n1-2as) for ASABF- ϵ , and (n2-2s, n2-2as) for ASABF- ζ . PCR reaction was carried out according the thermal profile: 95 °C for 10 min, (95 °C for 15 s, 60 °C for 1 min) $\times$ 40 cycles. Template concentration was estimated by the number of cycles needed for SYBR Green fluorescence to cross a predetermined threshold. The cDNA sample most abundantly containing a tested template was selected by a preliminary experiment, and diluted 1–5⁷-fold in a 5-fold increase to prepare a standard curve and evaluate the range of quantification. Relative amounts of the ASABF transcripts were assessed using the standard curve. The transcript for a putative 'housekeeping' gene, the *A. suum* homologue of elongation factor 1 α (EF1 α), was also tested as a control, i.e. (As-EF1a-1s, As-EF1a-1as). In the same way, 28 S rRNA was also quantified using 100-fold-diluted samples as a loading control, i.e. (As-28S-1s, As-28S-1as). Finally, the amount of tested transcripts was normalized by that of 28 S rRNA and compared among samples.

Virtual Northern blot

Total RNA was reverse-transcribed as described above. The single-strand cDNA was non-specifically amplified by PCR using SMART cDNA synthesis kit (Clontech). The optimal number of PCR cycles was selected so as to remain in the exponential phase of amplification and prevent its reaching a plateau. The amplified PCR product was directly electrophoresed on a 1.2 % agarose slab gel and transferred on to a GeneScreen Plus[®] membrane (DuPont). To denature the transferred DNA, the membrane was soaked in 1.5 M NaCl and 0.5 M NaOH for 10 min and neutralized in 0.2 M Tris/HCl, pH 7.5. The DNA on the membrane was fixed using a FS-1500 UV cross-linker (Funakoshi, Tokyo, Japan). Hybridization was performed using ³²P-labelled probes. In addition to EF1 α , the small subunit of cytochrome *b*₅₅₈ (cybS) was also used as loading controls [16]. Parts of cDNA were amplified by PCR and used as templates for probes, i.e. (1a-5'f, 1a-1as) $\times$ (1a-2s, 1a-2as) for ASABF- α , (1b-5'f, 1b-1as) $\times$ (1b-

2s, 1b-2as) for ASABF- β , (1c-5'f, 1c-1as) $\times$ (1c-2s, 1c-2as) for ASABF- γ , (1d-5'f, 1d-1as) $\times$ (1d-2s, 1d-2as) for ASABF- δ , (n1-1s, n1-1as) $\times$ (n1-2s, n1-2as) for ASABF- ϵ , (n2-1s, n2-1as) $\times$ (n2-2s, n2-2as) for ASABF- ζ , (As-EF1a-1s, As-EF1a-1as) for EF1 α , and (cb558-sR, cb558-2as) for cybS. The results of hybridization were evaluated using a BAS-2500 Bio Imaging Analyzer (Fuji-film, Japan).

RESULTS

Novel members of ASABF

Multiplication of antimicrobial-peptide genes has been widely observed in various organisms, including *C. elegans* [6]. On the other hand, ASABF- α was the only member which has been purified from *A. suum* [4]. Thus we expected that novel members should be found by further exploration in *A. suum*. The cDNA encoding ASABF was cloned using three-step PCR amplification, i.e. cloning of the internal sequence using degenerate primers, 5'-RACE, and 3'-RACE. The PCR products generated in each step were subcloned and re-examined. Three novel members of ASABF-type antimicrobial peptides were detected. These are designated as ASABF- β , - γ and - δ (Figure 1). To distinguish from these novel members, the original ASABF [4] was renamed ASABF- α in what follows. In addition, two other members were detected in the parasite EST databases. Their entire cDNAs were cloned and they were confirmed as the novel members ASABF- ϵ and - ζ (Figure 1). Conclusively, six members of ASABF-type antimicrobial peptides were identified in *A. suum*. All of their transcripts were detected in the body wall separately isolated from nine individuals, suggesting that the genes encoding these six ASABFs were involved in a haploid genome.

These six members can be aligned with few gaps (Figure 1). The primary structure of the ASABF-type antimicrobial peptide family was characterized using the alignment. First, a secretory signal region was predicted at the N-terminus of all members. Secondly, a cysteine-array consisting of eight cysteine residues was completely conserved, and sequences were highly identical in

Figure 2 Tissue specificity and test for bacterial inducibility of ASABF and EF1α transcripts

Left-hand panel: quantitative RT-PCR. Bars indicate saline-injected (black bars) or heat-killed bacteria-injected individuals (grey bars). Seven individuals were tested for each experiment. The accumulation of ASABF transcripts was estimated as a value relative to that of 28 S ribosomal RNA (non-immunized body wall = 1). EF1α was tested as a putative ubiquitous housekeeping gene. Each data point represents the mean + S.E.M. Asterisks indicate a significant difference between bacteria-injected and saline-injected samples ($P < 0.05$). Upper right-hand panel: virtual Northern blot. A typical result is represented. Total RNA from each individual was reverse-transcribed and amplified as double-strand cDNA. The amplified cDNA was subjected to electrophoresis, membrane-blotting and hybridization. EF1α and cybS were tested as loading controls. Numbers below each panel represent the relative amount of loading cDNA. Abbreviations: BW, body walls. IT, intestines. UT, uteruses. OV, ovaries. S, saline-injected. B, bacteria-injected. Lower right-hand panel: detection of transcripts for ASABF-ε and ζ. A nested PCR was performed using following primer sets, i.e. (n1-1s, n1-1as) × (n1-2s, n1-2as) for ASABF-ε, and (n2-1s, n2-1as) × (n2-2s, n2-2as) for ASABF-ζ. This experiment was not quantitative.

this region. Thirdly, an insect/arthropod defensin consensus motif, consisting of six cysteine residues and one glycine residue, was conserved as observed in ASABF- α and *C. elegans* homologues [4,6,12]. Fourthly, a dibasic site, suggested to contribute to the C-terminal processing in ASABF- α [4], was also conserved in ASABF- β , - γ and - ζ (but not in ASABF- γ and - ε), although the C-terminal region was divergent in length, suggesting that some novel members may undergo the similar processing to that of ASABF- α . Fifthly, all mature peptides were predicted to be cationic at neutral pH, as observed in many antimicrobial peptides.

Evolutionary relationship

The genes encoding ASABF- α and an ASABF-type antimicrobial peptide in *C. elegans*, ABF-1, have a single intron at a conserved position [6]. The exon–intron junctions of novel members were determined. All members also have a single intron at the conserved position (Figure 1), suggesting that all six members of ASABF-type antimicrobial peptides were diversified from a common ancestor.

Tissue-specific gene expression

The relative expression of ASABF in various tissues was estimated using a quantitative RT-PCR (Figure 2, left-hand panel). The transcript for ASABF- α was detected in the body wall, uterus and ovary, but only faintly in the intestine. That of ASABF- β was mainly accumulated in the body wall and intestine, but not in the reproductive organs. The ASABF- γ transcript was almost exclusively found in the body wall. In contrast, the transcripts for ASABF- δ were detected in all tested tissues, i.e. the body wall, intestine, uterus and ovary. In addition, the level of transcripts for ASABF was also estimated by virtual Northern blots (Figure 2, upper right-hand panel). The results of the virtual Northern blots agree with those suggested by quantitative RT-PCR, except where the ASABF- γ transcript was more clearly detected in the intestine of bacteria-injected individuals. Both the quantitative RT-PCR and virtual Northern blots could not detect the transcripts for ASABF- ε and ASABF- ζ , because the accumulation of these transcripts was possibly under the level detectable by these methods. We next carried out more highly sensitive, but non-quantitative, nested RT-PCR (Figure 2, lower right-hand panel). The transcript of ASABF- ε was found at least in the body wall and ovary. The ASABF- ζ was detected in the body wall, intestine, uterus and ovary. Under the same condition, the transcripts for other members (ASABF- α , - β , - γ and - δ) were also found in all tested tissues (results not shown). In conclusion: (1) all ASABF genes were expressed in the body wall; (2) relative expression in other tissues was diversified among ASABF members.

Induction by bacterial injection

To test whether ASABFs increase as an immune response, the inducibility of ASABF gene expression by bacterial injection was examined (Figure 2, left-hand panel and upper right-hand panel). It is important to distinguish a non-specific innate immune response from a specific pathological change by pathogens. Therefore, *E. coli* OP50 was selected to be injected, because this bacterium is non-pathogenic, at least to *C. elegans*. The heat-killed *E. coli* OP50 or sterile saline was injected into the pseudocoelom. Tissues were collected 4 h after the injection. Quantitative RT-PCR revealed that the transcripts for all tested ASABFs significantly increased in

the body wall by bacterial injection (8.9, 9.2, 3.8, and 13.2 times for ASABF- α , - β , - γ , and - δ respectively; Figure 2, left-hand panel). In addition, the ASABF- δ transcript obviously increased also in the intestine (37.1-fold). No significant increase in any ASABF transcript was observed in the reproductive organs (uterus and ovary). Although the virtual Northern blots indicate similar results, ASABF- β and ASABF- γ transcripts also seemed to be up-regulated in the intestine (Figure 2, upper right-hand panel). In contrast, the accumulation of the transcript for a putative housekeeping gene, EF1 α , was not significantly affected, at least in the body wall, intestine and uterus. These results suggest that some ASABF transcripts are up-regulated in the body wall and intestine by bacterial injection.

DISCUSSION

In the present study we have first demonstrated that the transcripts for ASABF-type antimicrobial peptides are induced by bacterial injection. This result suggests that nematodes can recognize bacterial invasion in the pseudocoelom and actively trigger a humoral defence reaction. Interestingly, some very old (1898 [13], 1923 [14]) papers described the phagocytosis of bacteria by the coelomocytes (i.e. the isolated cells in the body cavity of nematodes) in *Ascaris*, also suggesting the ability of non-self recognition in nematodes. It is noteworthy that these observations are also consistent with our conclusion, although these old data should be re-examined. An increase in ASABF transcripts was detected within 4 h, suggesting that the induction of ASABF is an acute-phase immune response in *A. suum*. The transcripts for all six members of ASABF were detected in the body wall, but were more diversified in other tissues, suggesting that their physiological roles may also be diversified.

Some insect antimicrobial peptides are also induced by injection of viable or heat-killed microbes, mainly under the control of Rel transcriptional factors; this mechanism is common between insects and vertebrates [1]. However, no Rel-like genes have been detected in the *C. elegans* genome, suggesting that the mechanism to induce ASABF may be substantially different from that of insect antimicrobial peptides. Recently, the defect in the 'DBL-1'/TGF- β ' (Duffy-binding-like domain-1/transforming growth factor- β) pathway caused increased susceptibility to infection by *S. marcescens* [9]. In addition, a MAP kinase (mitogen-activated protein kinase) cascade involving 'NSY-1' (neuronal symmetry-1), 'SEK-1' [SAPK (stress-activated protein kinase)]/ERK (extracellular-signal-regulated kinase) kinase-1] and 'PMK-1' (a p38 MAP kinase orthologue) has been reported to be necessary for the resistance to the pathogen *Pseudomonas aeruginosa* PA14 and *Enterococcus faecalis*, and those authors mentioned that the downstream targets of this p38-MAP-kinase pathway involved in mediating the nematode defence to pathogens remain to be elucidated [15]. In future studies it will be important to elucidate how nematodes recognize non-self molecules in the pseudocoelomic fluid and transmit signals to tissues producing ASABFs.

We thank Dr Naotoshi Tsuji (National Institute of Animal Health, Tsukuba-shi, Ibaraki-ken, Japan) for providing *A. suum* and helpful suggestions on keeping the worms. This work was supported by the Bio Design Program (BDP-02-V-1-x) and Program for Promotion of Basic Research Activities for Innovative Biosciences, Japan.

REFERENCES

- Hoffmann, J. A. and Reichhart, J. M. (2002) *Drosophila* innate immunity: an evolutionary perspective. *Nat. Immunol.* **3**, 121–126

- 2 Fujita, T. (2002) Evolution of the lectin-complement pathway and its role in innate immunity. *Nat. Rev. Immunol.* **2**, 346–353
- 3 Kato, Y. (1995) Humoral defense of the nematode *Ascaris suum*: antibacterial, bacteriolytic and agglutinating activities in the body fluid. *Zool. Sci.* **12**, 225–230
- 4 Kato, Y. and Komatsu, S. (1996) ASABF, a novel cysteine-rich antibacterial peptide isolated from the nematode *Ascaris suum*: purification, primary structure and molecular cloning of cDNA. *J. Biol. Chem.* **271**, 30493–30498
- 5 Zhang, H., Yoshida, S., Aizawa, T., Murakami, R., Suzuki, M., Koganezawa, N., Matsuura, A., Miyazawa, M., Kawano, K., Nitta, K. and Kato, Y. (2000) *In vitro* antimicrobial properties of recombinant ASABF, an antimicrobial peptide isolated from the nematode *Ascaris suum*. *Antimicrob. Agent. Chemother.* **44**, 2701–2705
- 6 Kato, Y., Aizawa, T., Hoshino, H., Kawano, K., Nitta, K. and Zhang, H. (2002) *abf-1* and *abf-2*, ASABF-type antimicrobial peptide genes in *Caenorhabditis elegans*. *Biochem. J.* **361**, 221–230
- 7 Zhang, H. and Kato, Y. (2003) Common structural properties specifically found in the CS α β -type antimicrobial peptides in nematodes and mollusks: evidence for the same evolutionary origin? *Dev. Comp. Immunol.*, in the press
- 8 Bányai, L. and Patthy, L. (1998) Amoebapore homologs of *Caenorhabditis elegans*. *Biochim. Biophys. Acta* **1429**, 259–264
- 9 Mallo, G. V., Kurz, C. L., Couillault, C., Pujor, N., Granjeaud, S., Kohara, Y. and Ewbank, J. J. (2002) Inducible antibacterial defense system in *C. elegans*. *Curr. Biol.* **12**, 1209–1214
- 10 Hodgkin, J., Kuwabara, P. E. and Corneliusson, B. (2000) A novel bacterial pathogen, *Microbacterium nematophilum*, induces morphological change in the nematode *C. elegans*. *Curr. Biol.* **10**, 1615–1618
- 11 Bulet, P., Cociancich, S., Dimarcq, J.-L., Lambert, J., Reichhart, J.-M., Hoffmann, D., Hetru, C. and Hoffmann, J. A. (1991) Insect immunity: isolation from a coleopteran insect of a novel inducible antibacterial peptide and of new members of the insect defensin family. *J. Biol. Chem.* **266**, 24520–24525
- 12 Cociancich, S., Goyffon, M., Bontems, F., Bulet, P., Bouet, F., Menez, A. and Hoffmann, J. (1993) Purification and characterization of a scorpion defensin, a 4 kDa antibacterial peptide presenting structural similarities with insect defensins and scorpion toxins. *Biochem. Biophys. Res. Commun.* **194**, 17–22
- 13 Nasonov, N. (1898) Sur les organes phagocytaires des ascarides. *Arch. Parasitol.* **1**, 170–179
- 14 Metchnikoff, S. I. (1923) Les quatre phagocytes d'*Ascaris megalcephala* et leur rôle dans l'immunité. *Ann. Inst. Pasteur* **37**, 680–685
- 15 Kim, D. H., Feinbaum, R., Alloing, G., Emerson, F. E., Carsin, D. A., Inoue, H., Tanaka-Hino, M., Hisamoto, N., Matsumoto, K., Tan, M.-W. and Ausubel, F. M. (2002) A conserved p38 MAP kinase pathway in *Caenorhabditis elegans* innate immunity. *Science* **297**, 623–626
- 16 Saruta, F., Hirawake, H., Takamiya, S., Ma, Y. C., Aoki, T., Sekimizu, K., Kojima, S. and Kita, K. (1996) Cloning of a cDNA encoding the small subunit of cytochrome *b*₅₅₈ (cybS) of mitochondrial fumarate reductase (complex II) from adult *Ascaris suum*. *Biochim. Biophys. Acta* **1276**, 1–5

Received 17 December 2002/10 February 2003; accepted 5 March 2003

Published as BJ Immediate Publication 5 March 2003, DOI 10.1042/BJ20021948