Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 323 (2004) 104–111
BBRC
www.elsevier.com/locate/ybbrc

ATP-binding cassette protein E is involved in gene transcription and translation in *Caenorhabditis elegans*

Zhongying Zhao*, Lily Li Fang, Robert Johnsen, David Leonard Baillie

Departments of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC, Canada V5A 1S6

Received 5 August 2004
Available online 27 August 2004

Abstract

ATP-binding cassette protein E (ABCE) gene has been annotated as an RNase L inhibitor in eukaryotes. All eukaryotic species show the ubiquitous presence and high degree of conservation of ABCEs, however, RNase L is present only in mammals. This indicates that ABCEs may function not only as RNase L inhibitors, but also may have other functions that have yet to be determined. As an initial investigation into the novel functions of ABCE, we characterized the gene (Y39E4B.1) in *Caenorhabditis elegans* by a combination of data mining and functional assays. ABCE promoters drove GFP expressions in hypoderm, pharynx, vulvae, head, and tail neurons at all developmental stages. Three genes, *rpl-4*, *nhr-91*, and C07B5.3, were previously found to interact with ABCE. Our expression data showed overlapping expression patterns of ABCE and *rpl-4* and *nhr-91*, but not C07B5.3. RNAi against ABCE resulted in embryonic lethality and slow growth. These data suggest that ABCE protein might be involved in the control of translation and transcription, work as shuttle protein between cytoplasm and nucleus, and possibly as a nucleocytoplasmic transporter. In addition, RNAi data suggest that ABCE and NHR-91 may function in vulvae development and molting pathways in *C. elegans*. Furthermore, our data suggest that ABCE, along with its interacting components, functions in a well-conserved pathway.
© 2004 Elsevier Inc. All rights reserved.

Keywords: ABC transporter; ABCE; RNase L inhibitor; *Caenorhabditis elegans*; Protein translation; Gene transcription

ATP-binding cassette (ABC) proteins constitute one of the largest protein families in both prokaryotes and eukaryotes. Most ABC proteins are transporters and are involved in the transport of various molecules across plasma membranes and intracellular membranes of organelles like endoplasmic reticulum, peroxisome, and mitochondria [1,2]. A functional ABC transporter generally consists of at least one evolutionarily conserved ABC domain, also known as a nucleotide binding domain (NBD) with approximately 200 amino acids, and several α-helical transmembrane domains (TMD). The NBD contains three conserved domains: Walker A and B motifs which can be found in all ATP-binding proteins, and a signature (C) motif, located just upstream of the Walker B site [3]. The C motif is diagnostic of ABC transporters and distinguishes them from other ATP-binding proteins. Twenty-nine yeast ABC transporter genes were reported in yeast [4]. Sixty ABC transporter genes have previously been identified in the nematode *Caenorhabditis elegans*' genome [5]. They are classified into eight subfamilies, i.e., ABCA to ABCH. Many of these subfamilies include multiple duplicated paralogues. However, only one member of the ABCE subfamily, i.e., Y39E4B.1, has been identified in *C. elegans*. Unlike typical ABC proteins, the ABCE protein, together with ABCF proteins, contains two NBDs but no TMD, suggesting it is not a transmembrane transporter. Sequences of ABCE proteins are highly conserved among all eukaryotic species, especially in their NBD domains, which show over 90% identity

* Corresponding author. Fax: +1 604 291 5583.
E-mail address: zhao@gene.mbb.sfu.ca (Z. Zhao).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.08.068

across all eukaryotes [6]. Similar to *C. elegans*, ABCE has been found to have only one subfamily member in most eukaryotes, except in *Arabidopsis thaliana* (Table 1). No obvious ABCE homologues were found in bacteria [6], suggesting its fundamental role in eukaryotes.

ABCE genes of eukaryotes are commonly annotated as RNase L inhibitor which was first identified in human [7]. It is implicated in the antiviral mechanisms of IFN and might regulate RNA turnover and stability [8]. IFN induces four different forms of human 2–5A-synthetase which, upon activation by double-stranded RNA (dsRNA), converts ATP into an unusual series of oligomers known as 2–5A. 2–5A-dependent RNase L is the effector enzyme of this system. Its activation by subnanomolar levels of 2–5A leads to the inhibition of protein synthesis by cleavage of mRNA at the 3′ side of UpNp sequences [9–11]. RNase L appears to have homologues only in mammals such as human, mouse, and rat. No apparent RNase L homologues have been identified in other eukaryotes or in the archaea which possessed a candidate ABCE gene [6]. Classically, ABCE's (present in all eukaryotic organisms) main function was thought to be as an RNase L inhibitor. However, most eukaryotic organisms do not contain RNase L, therefore, the ABCE genes very likely have other functions. The search for the novel functions of these proteins constitutes the main focus of this paper.

Caenorhabditis elegans as a model system provides an opportunity to study ABCE functions within the context of a developing multicellular organism. As an initial step into identifying ABCE's functions, we characterized this gene in *C. elegans* by a combination of data mining and expressional and functional assay. Like in rice [12], ABCE is constitutively expressed in *C. elegans*. Our results suggest that ABCE is involved in translation and transcriptional control, regulation of growth and vulvae development, and very possible functions in well-conserved pathways among all eukaryotes.

Table 1
Conservation of ABCE across eukaryotes

	Protein length	Length of aligned region[a]	Identity (%)	No. of paralogs
C. elegans	610	610	100	1
C. briggsae	610	610	95	1
D. melanogaster	611	603	67	1
M. musculus	599	594	66	1
H. sapiens	599	594	65	1
S. cerevisiae	608	605	63	1
A. thaliana[b]	605	599	65	2
C. elegans[c]	1431	169	24	7 [5]

Protein sequences are retrieved from GenBank or EMBL, aligned against *C. elegans* ABCE protein sequence by online pairwise BLAST program from NCBI with default parameters except the filter is turned off (see Materials and methods).

[a] Alignment against *C. elegans* sequence.

[b] The sequence used for alignment is TrEMBL: Q8LPJ4 otherwise retrieved from GenBank.

[c] *C. elegans* homologue (F55G11.9) with highest identity to ABCE.

Materials and methods

Strains. All strains were maintained and cultured using standard techniques. N2 (Bristol); KR3532, dpy-5(e907); GR1373 *eri-1*(*mg366*) IV; BC10798, dpy-5(e907), sEX10798[dpy-5(+) + rCesY39E4B.1-GFP + pCes361]; BC06518, dpy-5(e907), sEX1050[dpy-5(+) + rCes rpl-4::GFP + pCeh361]; BC06519, dpy-5(e907), sEX1051[dpy-5(+) + rCesC07B5.3-GFP + pCes361]; BC06495, dpy-5(e907), sEX1029[dpy-5(+) + rCes-nhr-91-RFP + pCes361]; BC12778, dpy-5(e907), sEX12778[dpy-5(+) + rCesC26C6.5-GFP + pCes361]; BC11724, dpy-5(e907), sEX11724[dpy-5(+) + rCesF36H1.2-GFP + pCes361].

Sequence analysis and data mining. All protein sequences were derived from NCBI, EMBL or Wormbase (WS126). Pair-wise alignments against *C. elegans* ABCE protein sequence were done with NCBI online pairwise BLASTP program: BLAST 2 SEQUENCES using the default parameters except with "Filter" turned off. The ABCE protein sequences from different species were also subjected to multiple alignment using CLUSTALX [13] with Gonnet protein weight matrix. SAGE data were retrieved from BC Genome Science Center site: http://elegans.bcgsc.bc.ca.

Construction of promoter:reporter fusion PCR products. Promoter or full-length green fluorescence protein/red fluorescence protein (GFP/DsRed) fusion constructs were built basically as described [14]. GFP coding sequence for promoter fusion was derived from A. Fire's vector pPD-95-67. The primer sequences used for GFP amplification are exactly the same as those used previously [14]. Similar C, D, and D* primers were used to amplify DsRed with sequences: 5′-CGC TCA TCA AGA GAA AAA TGG-3′, 5′-AAA CGC GCG AGA CGA AAG-3′, and 5′-GGAAACAGTTATGTTTGGTATATTGGG-3′, respectively. The full-length protein fusion with DsRed was generated using similar methods, i.e., by fusion PCR [14] using the same D and D* primers but a different C primer sequence unique to DsRed: CCA TTT TTC TCT TGA TGA GCG G.

Microinjection. Dpy-5 gene was used as a selectable marker to screen for transgenic worms. The *dpy-5* rescuing plasmid, pCes-361 (kindly provided by Dr. Ann Rose and Dr. Colin Thacker), was co-injected with a fusion construct into *dpy-5* (e907) mutant worms at concentrations of 100 and 10 ng/μl, respectively. Wild type F1 worms, after a 3-day, 20 °C incubation were transferred to a fresh NGM plate and after another 3-day, 20 °C incubation, were scored for wild type progeny to isolate a heritable transgenic line.

Microscopy. The transgenic lines were first examined for GFP or RFP expression using a ZESIS Stemi SV11 dissecting microscope with GFP and RFP filters. All pictures were taken with a QIMAGING digital camera mounted on a ZESIS Axioskop compound microscope. Of the dozens of worms photographed, only those showing the same expression patterns were counted due to the mitotic loss of some transgenes.

RNAi using eri-1(mg366) mutant [15]. Given the expression of ABCE and its interacting proteins in neurons, RNAi feeding was done with mutant *eri-1*(*mg366*) as described previously [16]. RNAi bacteria (kindly provided by Dr. J. Ahringer) were streaked onto NGM plates containing ampicillin, tetracycline, and IPTG, and incubated overnight at room temperature. Ten L4 *eri-1*(mg366) worms were seeded onto the plates and incubated at room temperature for another 24 h. Three (*eri-1mg366*) worms were transferred onto the three replica plates and scored for RNAi phenotypes every 8 h for 3 consecutive days. The RNAi worms were subjected to second round RNAi using the same bacteria to score the phenotypes.

Results

ABCEs are well conserved across all eukaryotic species

The protein sequences of eukaryotic ABCE are well conserved. There is over 65% identity between human and worm ABCE protein sequences. They also have comparable numbers of amino acids in each protein, ranging from 594 to 610 (Table 1). Surprisingly, the ABCE subfamily has only one member in most species except for *A. thaliana*, which has two members (Table 1). This indicates there have been few gene duplications or that, in general, no functional redundancy, or separable noval function ABCE proteins have evolved. The *C. elegans* homologue with highest similarity to ABCE is a member of the ABCF subfamily, i.e., F55G11.9. However, the percentage of identity between worm ABCE and F55G11.9 is significantly lower than that between worm ABCE and its orthologues. In addition, F55G11.9 encodes 1431 as opposed to 610 amino acids for worm ABCE and only 169 amino acids can be aligned between worm ABCE and F55G11.9 (Table 1). The ubiquitousness and high degree of conservation suggest the significant functions of ABCE among all eukaryotes.

ABCEs function as more than RNase L inhibitors

ABCEs in all eukaryotic species are commonly annotated as RNase L inhibitors because they were first isolated from an expression library by binding to 2–5ApCp and associated with and inhibited RNase L [7]. Given the ubiquitous nature and high conservation of ABCEs across eukaryotes, we expect the proteins involved in the same pathway with ABCE to be conserved and co-localized. However, BLAST searches of the non-redundant sequence database identified hits only in human, mouse, and rat sequence, using human ABCE sequence as a query, and employing low complexity filtering to mask the ankyrin repeats of RNase L. No putative RNase L homologues were identified in any eukaryotes in which the ABCE gene was identified [6]. From this we argue against ABCE functioning as an RNase L inhibitor in eukaryotes because RNAse L is absent in species in which ABCE is present. Presence of RNase L in mammals suggests an immune system which has evolved to fight viruses unique to these species. So ABCE may function partly in antiviral pathway in mammals, the remaining functions in all eukaryotes having yet to be determined.

Expression patterns of ABCE in C. elegans

As an initial step in investigating the role of ABCE, a promoter::GFP fusion construct was generated by PCR (see Materials and methods). The promoter contained a 2965 bp sequence upstream of the start ATG. The resulting PCR product was co-injected with *dpy-5*(+) plasmid into dpy-5 hermaphrodite to generate a transgenic line. The 2965 bp ABCE promoter drove GFP expressions in the majority of tissues and developmental stages, including hypoderm, anterior and posterior neurons, muscle, pharynx, and vulva in adults, larvae, and embryos (Fig. 1 and Table 2). The worm homologue with the lowest *E* value for human RNase L is ankyrin protein (F36H1.2), with an unknown function. Similar promoter::GFP transgenic lines were generated for the F36H1.2. It expressed mostly in the excretory cell and the intestine, but it had few overlapping patterns as those of ABCE (Table 2), suggesting different pathways for the two genes in *C. elegans*.

Proteins that interact with ABCE are completely or partially co-localized

A large scale yeast two-hybrid screen was done in *C. elegans* [17], in which several proteins (RPL-4, NHR-91, and C07B5.3) were found to interact directly with the worm ABCE protein. This significantly advanced our understanding of the potential functions of ABCE. The interactions of ABCE with two of the three proteins were confirmed by our transgenic report assay which showed that worm ABCE is completely or partially co-expressed with rpl-4, nhr-91 but not C07B5.3 (Fig. 1). Both ABCE and RPL-4 can be seen in hypoderm, muscle, pharynx, and neurons (Fig. 1). *rpl-4* encodes a large ribosomal subunit L4 protein. Yeast RPL4 may be one of the first ribosomal proteins that bind to the 35S pre-rRNA molecule during ribosome biogenesis [18]. The interaction and co-expression of ABCE with RPL-4 strongly suggests that ABCE is involved in protein translation control. This is consistent with observations that human ABCE interacts with the initiation factor 2 [19]. Lower number of SAGE tags for ABCE than for *rpl-4* (Table 2) suggests that ABCE is not a constitutive part of ribosomes but more likely a regulatory component. The exact biochemical mechanisms of ABCE in protein translation regulation require further investigation.

nhr-91 is a member of the nuclear receptor (NR) superfamily. This family has undergone a dramatic expansion and diversification in *C. elegans* but not in other phyla [20]. The complete *C. elegans* genome contains 284 confirmed or predicted NR genes, over 5-fold more than the number found in the human or *Drosophila melanogaster* genomes [21]. Among these 284 NR genes, 15, including *nhr-91*, are conserved among the metazoa [22]. A promoter: DsRed construct of *nhr-91* gave expression in embryos, larvae, and adults. DsRed was observed in adult head and tail neurons, hypoderm, vulvae, seam cells, and excretory duct cell. These observations were consistent to what was reported previously

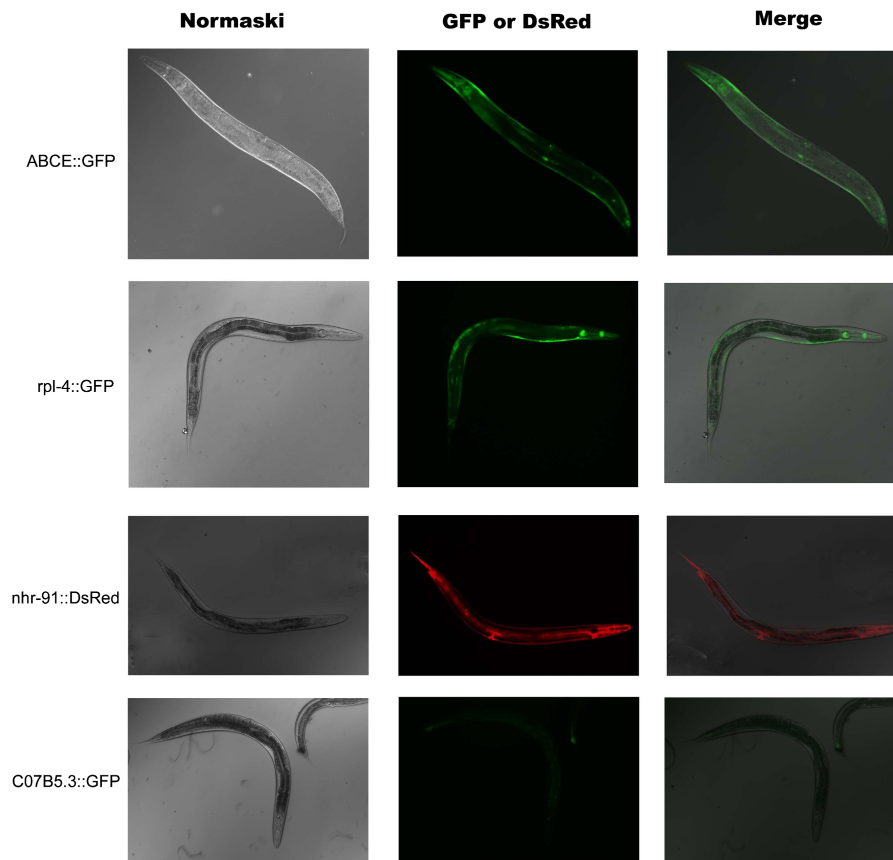

Fig. 1. Localization of ABCE and its interacting components by transgenic report assay. Normaski and fluorescent pictures were taken and superimposed to each other. ABCE promoter drove GFP expressions in hypoderm, vulvae, muscle, pharynx, head, and tail neurons. Rpl-4 promoter drove GFP expressions in hypoderm, muscle, pharynx as well as head, and tail neurons. Nhr-91 promoter drove DsRed expressions in hypoderm, vulvae, seam cell, excretory duct cell as well as head and tail neurons. C07B5.3 promoter only drove weak GFP expressions in posterior intestine. Overlapping expression patterns were observed between ABCE, rpl-4, and nhr-91, but not C07B5.3.

Table 2
Comparison of expression pattern and RNAi phenotypes of Y39E4b.1 and its related genes

Gene name	Expression patterns	RNAi phenotypes	No. of SAGE tags[c]
Y39E4B.1	Hypoderm, muscle, pharynx, neurons, vulva	Emb[d] Gro[e]	1
rpl-4[a]	Hypoderm, muscle, pharynx and neurons	Emb Ste[f]	23
nhr-91[a]	Neurons, vulva, seam cells, excretory duct cell	WT[g] **Emb Gro Vul**[k]	0
C07B5.3[a]	Weak in posterior gut	WT	0
F36H1.2[b]	Intestine, excretory cell	Dpy[h] Pvl[i] Rup[j]	3

[a] Genes that interact with Y39E4B.1 [16].
[b] Highest score BLASTP hit in WormPep using human RNAse L sequence as query.
[c] Number of long SAGE tags in embryo [26].
[d] Embryonic lethality.
[e] Slow growth.
[f] Sterile.
[g] Wild type.
[h] Dumpy.
[i] Protruding vulva.
[j] Exploded body.
[k] Phenotypes (in bold font) were observed by RNAi with *eri-1* mutant only.

[22] (Table 2). Expression was also observed in larval intestine. Both ABCE and *nhr-91* expressed in vulvae, hypoderm, and neurons (Fig. 1). Interaction and co-expression of ABCE and *nhr-91* suggests that the two genes are involved in the same conserved pathway. Since NHR-91 is a transcription factor interaction of ABCE protein and NHR-91 suggests the potential role of ABCE in gene transcription.

Given the interaction of ABCE protein with both NHR-91 and RPL-4, it is possible that ABCE may function not only in protein translation control, but also in communications between gene transcription and translation, possibly encoding a shuttle protein between cytoplasm and nucleus. Since both the ABCE protein and NHR-91 are well conserved across species, it is plausible that the pathway in which the two proteins are involved should also be conserved across all other eukaryotic species.

No obvious orthologues were identified for C07B5.3 in other phyla (data not shown), suggesting its unique roles in nematodes. It is expressed weakly in adult posterior gut (Fig. 1). Few overlapping expression patterns were seen between ABCE and C07B5.3, suggesting a

possible false positive interaction between the two gene products in yeast two-hybrid [17]. But this is a tentative conclusion because our GFP expression data for C07B5.3 are inconsistent with previous in situ hybridization patterns [23]. We have yet to determine the reason for the discrepancies.

We tried generating full-length protein fusion of ABCE (including 2.7 kb of its upstream sequence) and DsRed. The fusion construct was too toxic to get successful transgenic lines, implying overproduction of ABCE causes lethality during worm development.

RNAi using eri-1 mutant captured extra phenotypes for nhr-91

Both ABCE and RPL-4 are essential for normal development based on existing RNAi data [16]. In order to further investigate the potential roles of ABCE and its interacting proteins, RNAi was done for ABCE and its interacting proteins using *eri-1*(*mg366*) due to its RNAi susceptibility in nerve systems (Table 2) [15]. In addition to the phenotypes previously observed, additional phenotypes were observed for *nhr-91* (Fig. 2, Table 2 in bold font). The progeny of worms after RNAi against *nhr-91* showed severely slow growth: 70% of eggs developed into L2 to L4 stage after 3 day incubation on RNAi plates in 20 °C. In addition, slight embryonic lethality was also observed. Slow growth and embryonic lethality suggest that NHR-91, together with ABCE protein, are involved in the transcription control of genes involved in development and growth. Microscopic examination of the morphology of slow-growing worms demonstrated that many of them had defects in vulvae development and molting (Figs. 2A and B). Embryonic lethality was also observed as previously seen [16,24]. Some worms produced few dead embryos inside the uterus (Fig. 2C). Expression of both ABCE and NHR-91 in vulvae and hypoderm suggests that two proteins function in molting and vulvae development pathways in *C. elegans*. Cyst was also observed around the intestine (Fig. 2D).

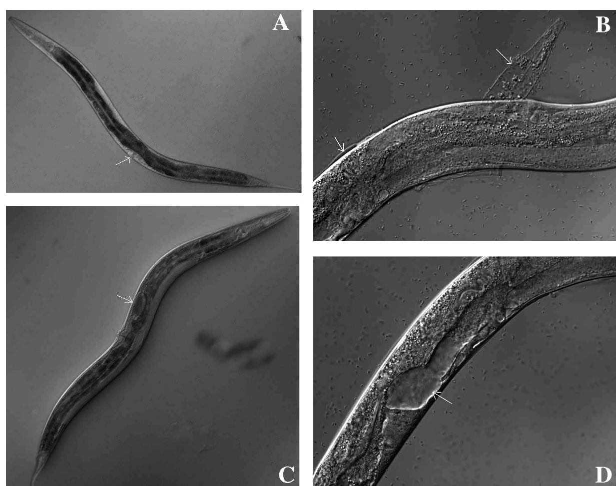

Fig. 2. RNAi phenotypes for *nhr-91*. RNAi against nhr-91 was done with *eri-1* mutant for consecutive two generations (see text). (A) A slow-growing worm after 3 day incubation at 20 °C, underdeveloped vulvae was shown. (B) Some worms showed defects in molting. (C) A dead embryo was shown inside the uterus of a young adult. (D) Cysts were observed around the intestine for a RNAied young adult.

Discussion

ATP-binding cassette protein E protein has been annotated as RNase L inhibitor in all sequenced eukaryotes because it has been demonstrated in vitro that this protein reversibly associates and inhibits RNase L [7]. This is probably not biologically significant for non-mammalian species because only mammals have RNase L. All eukaryotic species show a high degree of conservation of ABCEs. This indicates that ABCEs have functions other than as RNase L inhibitors.

As an initial step in investigating the potential roles of ABCE in other eukaryotic organisms, we performed both in silico and functional experiments to identify roles in which ABCE might be involved. Scanning *C. elegans* ABCE protein sequence with Pfam yields four domains, which include a possible metal-binding domain, a 4Fe–4S-binding domain as well as two ABC domains from the N-terminal to the C-terminal (Fig. 3A). The full-length protein sequences of ABCEs are well conserved eukaryotes (Fig. 3B, Table 1). The function of the metal-binding domain remains to be determined. Proteins containing 4Fe–4S-binding domains include bacterial ferredoxins, various dehydrogenases, and various reductases. It is speculated that the 4Fe–4S-binding domain may mediate the interaction with DNA or RNA due to the two conserved positively charged lysines within the domain [25]. Two ABC domains are well conserved across all species. However, the absence of transmembrane domains makes ABCE unlikely to be a transmembrane transporter. These transmembrane domains are typical for most ABC transporter proteins.

Its extremely high conservation and lack of paralogues (Table 1) suggest ABCE plays an essential role among all eukaryotic species. This was supported by RNAi data in *C. elegans*, i.e., embryonic lethality. The fusion PCR products of the ABCE promoter with either GFP or RFP are so toxic that their concentrations in injection mixture had to be diluted to 100 times lower (100 pg/μl) than those normally used for regular micro-injection, i.e., 10 ng/μl (data not shown), suggesting the essential functions of ABCE. This implies that transcription factors might be taken by the transgenic array, thus are not available to activate the endogenous expression of ABCE. Lack of sufficient endogenous ABCE expressions might lead to problems for normal worm growth.

Fig. 3. ABCE proteins are well conserved across species. (A) Schematic representation of domain organization for ABCE based on Pfam annotations. *C. elegans* ABCE protein contains 610 residues. Residue 17–48 (red) at N-terminus is annotated as metal-binding domain, 59–68 as 4Fe–4S binding domain (blue), and 114–303 and 381–548 as two ABC domains (pink). (B) ABCE protein sequences from different species are aligned by CLUSTALX (see Materials and methods). Except for *A. thaliana*, there is only one ABCE in each species. The GeneDoc was used to edit the resulted alignment. The corresponding domains in (A) are boxed accordingly. Sequences are strongly conserved not only for annotated domains, but also for sequences outside these domains.

Three components were found to interact with ABCE protein (Y39E4B.1), namely, RPL-4, NHR-91, and C07B5.3 [17]. Co-localization of ABCE with two out of the three proteins confirms the interaction of ABCE protein and RPL-4 and NHR-91. *rpl-4* encodes a large ribosomal subunit L4 protein. About 2/3 of the mass of the ribosome consists of RNA and 1/3 of protein. The proteins are named in accordance with the subunit of the ribosome to which they belong—the small (S1 to S31) and the large (L1 to L44). RPL-4 is the number four protein of the large ribosomal subunit and may be involved in direct binding of rRNA because it is the most basic ribosomal protein [26]. RPL-4 was demonstrated in yeast as one of the first ribosomal proteins that bind to the 35S pre-rRNA molecule during ribosome biogenesis [18]. Human RPL-4 has been shown to aggregate in importin free cytoplasm and import of RPL-4 to nucleus is Ran-GFP dependent [26]. Given

the interaction of ABCE with RPL-4, a cytoplasmic component, and NHR-91, a nuclear transcription factor, we speculate that ABCE might also be involved in the transport of the two proteins in opposite directions in an ATP dependent way. Since the RPL-4 protein is a component of ribosome, a protein translation machinery, the interaction and co-localization of ABCE protein with RPL-4 suggests that ABCE is involved in protein translation control through RPL-4. The observations that human ABCE interacts with initiation factor 2 also suggest ABC's roles in protein translation [19]. However, low level expression of ABCE compared to that of RPL-4 by SAGE analysis data [27] suggests that ABCE functions not as a constitutive component of ribosomes also as a regulator. The exact mechanism of ABCE in protein translation regulation needs to be further investigated. *nhr-91* is a member of the nuclear receptor (NR) superfamily which encodes the zinc finger transcription factor. The NR superfamily has undergone a dramatic expansion and diversification in *C. elegans* and this not been observed in other phyla [20]. The completed *C. elegans* genome contains 284 confirmed or predicted NR genes, over 5-fold more than the number found in the human or *D. melanogaster* genomes [21]. Among these 284 NR genes 15 are conserved among the metazoan. *Nhr*-91 is one of these 15 conserved NR genes [22], suggesting its significant roles in gene expression regulation. Interaction and co-expression of ABCE and NHR-91 (Fig. 1) suggests that the two genes are involved in the same conserved pathway of gene expression regulation in *C. elegans*.

All NRs share a common structure with specific, conserved domains that function in DNA binding, ligand binding, and transcriptional activation [28]. The exact mechanisms of NHR-91 in transcriptional regulation remain to be determined, but because of its conservation across species, it is reasonable to speculate that NHR-91may function as a transcription factor in a conserved pathway [22]. Given the interaction of ABCE protein and NHR-91, in addition to protein translation control, ABCE may also function in transcription regulation and communications between gene transcription and translation, possibly mediating feedback regulation between two gene transcription and translation because of interaction with both cytoplasmic protein RPL-4 and nuclear protein NHR-91. This hypothesis is supported by PSORT [29] prediction of worm ABCE: 37% possibility in nucleus and 20% in cytoplasm, suggesting it be a shuttle protein between cytoplasm and nucleus. Evidences from conserved co-regulation are also in agreement with the hypothesis. For example, *Saccharomyces cerevisiae* and *C. elegans* ABCEs are both co-expressed with a number of proteins involved in the processing of rRNA such as the nucleolar protein SIK1 (NOP56) from yeast that is involved in rRNA methylation [30], implying ABCE's role in ribosome biogenesis (protein translation). Genomic context data and homology also predict the possible functions for ABCE in both translation and transcription. Among the 44 orthologous groups, which have an identical phylogenetic distribution with ABCE, most of them are either involved in translation or ribosome biogenesis (33, 60%), in transcription (7, 16%), and in DNA replication, recombination, and repair (3, 7%) [25]. Recent investigation of mRNP movement from the chromatin to the nuclear pore showed that there is an active component involved in the nuclear motion of these mRNP particles, and also that mRNP mobility is ATP dependent [31]. Combining this with our expression data, we suggest that ABCE is involved in the transport of mRNA from nucleus to cytoplasm.

nhr-91 RNAi using *eri-1*(*mg366*) strain gave significantly slower-growing worms than those with either wild type N2 strain or the rrf-3 strain. Expression of both ABCE and *nhr-91* was seen both in hypoderm and vulvae. RNAi with *nhr-91* on *eri-1* mutant worms also causes defects in vulvae development and molting. The external surface of *C. elegans* is formed by hypodermis which establishes the basic body form of the animal, acts in nutrient storage, secretes the cuticles, and takes up apoptotic cell bodies by phagocytosis. Together, the two genes may function together in vulvae development and molting pathway. Phenotypes captured using *eri-1* but not N2 suggest functional association of NHR-91 with neurons. The underlying mechanisms of the association need to be further investigated.

No obvious orthologues were identified for C07B5.3 other than in nematode (data not shown), indicating it has unique roles in nematodes. It showed weak expressions in adult gut, which is different from what was reported previously [23]. It remains to be determined what causes the discrepancy. Based on our data, we believed that interaction of ABCE and C07B5.3 is false positive result from yeast two-hybrid screening due to lack of overlapping expression data between the two genes.

Like ABCE, ABCF proteins contain two ABC domains but no transmembrane domains. It has been proposed that ABCF is involved in translation initiation and elongation [6]. Investigation of functional correlation of the structurally related ABCE and F proteins will provide new insights the roles of both subfamilies in eukaryotic species.

Acknowledgments

We thank Dr. Ann Rose and Colin Thacker for *dpy-5* rescuing plasmid and mutant worms, Dr. Andy Fire for GFP vector, SAGE data, Dr. J.A. Ahringer for RNAi bacteria, and the Caenorhabditis Genetics Center for *eri-1*(mg366) strain. This work depended crucially upon free access to the *Caenorhabditis elegans* sequence data in GenBank and WormBase. We express our gratitude

to Martin Jones for help during RNAi and Christine Beauchamp for critical proofreading of the manuscripts. This project was funded by NSERC Canada and Genome British Columbia/Genome Canada.

References

[1] S. Childs, V. Ling, The MDR superfamily of genes and its biological implications, Important Adv. Oncol. (1994) 21–36.
[2] M. Dean, R. Allikmets, Evolution of ATP-binding cassette transporter genes, Curr. Opin. Genet. Dev. 5 (1995) 779–785.
[3] S.C. Hyde, P. Emsley, M.J. Hartshorn, M.M. Mimmack, U. Gileadi, S.R. Pearce, M.P. Gallagher, D.R. Gill, R.E. Hubbard, C.F. Higgins, Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport, Nature 346 (1990) 362–365.
[4] A. Decottignies, A. Goffeau, Complete inventory of the yeast ABC proteins, Nat. Genet. 15 (2) (1997) 137–145.
[5] J.A. Sheps, S. Ralph, Z. Zhao, D.L. Baillie, V. Ling, The ABC transporter gene family of *C. elegans* has implications for the evolutionary dynamics of multidrug resistance in eukaryotes, Genome Biol. 5 (2004) R15.
[6] I.D. Kerr, Sequence analysis of twin ATP binding cassette proteins involved in translational control, antibiotic resistance, and ribonuclease L inhibition, Biochem. Biophys. Res. Commun. 315 (1) (2004) 166–173.
[7] C. Bisbal, C. Martinand, M. Silhol, B. Lebleu, T. Salehzada, Cloning and characterization of a RNase L inhibitor. A new component of the interferon-regulated 2–5A pathway, J. Biol. Chem. 270 (1995) 13308–13317.
[8] B.A. Hassel, A. Zhou, C. Sotomayor, A. Maran, R.H. Silverman, A dominant negative mutant of 2–5A-dependent RNase suppresses antiproliferative and antiviral effects of interferon, EMBO J. 12 (1993) 3297–3304.
[9] G. Floyd-Smith, E. Slattery, P. Lengyel, Interferon action: RNA cleavage pattern of a (2′–5′) oligoadenylate-dependent endonuclease, Science 212 (1981) 1030–1032.
[10] I.M. Kerr, R.E. Brown, pppA2′p5′A2′p5′A: an inhibitor of protein synthesis synthesized with an enzyme fraction from interferon-treated cells, Proc. Natl. Acad. Sci. USA. 75 (1978) 256–260.
[11] A. Zhou, B.A. Hassel, R.H. Silverman, Expression cloning of 2–5A-dependent RNase: a uniquely regulated mediator of interferon action, Cell 72 (1993) 753–765.
[12] X.L. Du, D. Wang, X.Y. Qian, L.Z. Jiang, W. Chun, K.G. Li, G.A. Shen, C.F. Lin, J.S. Yang, cDNA cloning and expression analysis of the rice (*Oryza sativa* L.) RNase L inhibitor, DNA Seq. 14 (4) (2003) 295–301.
[13] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucleic Acids Res. 24 (1997) 4876–4882.
[14] O. Hobert, PCR fusion-based approach to create reporter gene constructs for expression analysis in transgenic *C. elegans*, Biotechniques 32 (2002) 28–730.
[15] S. Kennedy, D. Wang, G. Ruvkun, A conserved siRNA-degrading RNase negatively regulates RNA interference in *C. elegans*, Nature 427 (6975) (2004) 645–649.
[16] R.S. Kamath, A.G. Fraser, Y. Dong, G. Poplin, R. Durbin, M., A. Kingpin, N. Le Boot, S. Moreno, M. Shoran, D.P. Welshman, L.P. Zipper, J. Harbinger, Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi, Nature 421 (6920) (2003) 231–237.
[17] S. Li et al., A map of the interactome network of the metazoan *C. elegans*, Science 303 (5657) (2004) 540–553.
[18] L.C. Yeh, J.C. Lee, Yeast ribosomal proteins L4, L17, L20, and L25 exhibit different binding characteristics for the yeast a35S precursor rRNA, Biochem. Biophys. Acta 1443 (1–2) (1998) 139–148.
[19] J.K. Tyzack, X. Wang, G.J. Belsham, C.G. Proud, ABC50 interacts with eukaryotic initiation factor 2 and associates with the ribosome in an ATP-dependent manner, J. Biol. Chem. 275 (44) (2000) 34131–34139.
[20] A. Sluder, S.W. Mathews, D. Hough, V.P. Yin, C.V. Maina, The nuclear receptor superfamily has undergone extensive proliferation and diversification in nematodes, Genome Res. 9 (1999) 103–120.
[21] J.M. Maglich, A. Sluder, X. Guan, Y. Shi, D.D. McKee, K. Carrick, K. Kamdar, T.M. Willson, J.T. Moore, Comparison of complete nuclear receptor sets from the human, *C. elegans* and *Drosophila* genomes, Genome Biol. 2 (2001) research 0029.1–0029.7.
[22] C.R. Gissendanner, K. Crossgrove, K.A. Kraus, C.V. Maina, A.E. Sluder, Expression and function of conserved nuclear receptor genes in *C. elegans*, Dev. Biol. 266 (2) (2004) 399–416.
[23] P.S. Birchall, R.M. Fishpool, D.G. Albertson, Expression patterns of predicted genes from the *C. elegans* genome sequence visualized by FISH in whole organisms, Nat. Genet. 11 (1995) 314.
[24] F. Simmer, C. Moorman, A.M. Van Der Linden, E. Kuijk, P.V. Van Den Berghe, R. Kamath, A.G. Fraser, J. Ahringer, R.H. Plasterk, Genome-wide RNAi of *C. elegans* using the hypersensitive rrf-3 strain reveals novel gene functions, PLoS Biol. 1 (1) (2003) E12 (Epub 2003).
[25] T. Gabaldon, M.A. Huynen, Prediction of protein function and pathways in the genome era, Cell Mol. Life Sci. 61 (7–8) (2004) 930–944.
[26] S. Jakel, J.M. Mingot, P. Schwarzmaier, E. Hartmann, D. Gorlich, Importations fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains, EMBO J. 21 (3) (2002) 377–386.
[27] S.J. McKay, R. Johnsen, J. Khattra, J. Asano, D.L. Baillie, S. Chan, N. Dube, L. Fang, B. Goszczynski, E. Ha, E. Halfnight, R. Hollebakken, P. Huang, K. Hung, V. Jensen, S.J.M. Jones, H. Kai, D. Li, A. Mah, M. Marra, J. McGhee, R. Newbury, A. Pouzyrev, D.L. Riddle, E. Sonnhammer, H. Tian, D. Tu, J.R. Tyson, G. Vatcher, A. Warner, K. Wong, Z. Zhao, D.G. Moerman, in: Gene Expression Profiling of Cells, Tissues and Developmental Stages of the Nematode *C. elegans*, Cold Spring Harbor Symposium LXVIII: The Genome of *Homo sapiens*, Cold Spring Harbor Press, Cold Spring Harbor, New York, 2003.
[28] H. Gronemeyer, V. Laudet, Transcription factors 3: nuclear receptors, Protein Profile 2 (1995) 1173–1308.
[29] Available from: <http://psort.nibb.ac.jp/form2.html>.
[30] V. van Noort, B. Snel, M.A. Huynen, Predicting gene function by conserved co-expression, Trends Genet. 19 (2003) 238–242.
[31] A. Calapez, H.M. Pereira, A. Calado, J. Braga, J. Rino, C. Carvalho, J.P. Tavanez, E. Wahle, A.C. Rosa, M. Carmo-Fonseca, The intranuclear mobility of messenger RNA binding proteins is ATP dependent and temperature sensitive, J. Cell Biol. 159 (2002) 795–805.