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Duplication of an Hsp70 gene in isolates of the colonizer nematode species *Acrobeloides nanus* may suggest genome plasticity

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Acrobeloides nanus is a dynamic colonizer nematode species, which exhibits high ecological plasticity. The purpose of this study was to investigate the genomic plasticity in *A. nanus* by examining the genomic divergence of a 70-kilodalton heat shock protein gene among different isolates of the species. Southern-blot hybridization experiments using as a probe the *hsp70-1* sequences of *Caenorhabditis elegans*, resulted in two different hybridization patterns for the *A. nanus* isolates. Pattern I was similar to that of *C. elegans*. Pattern II showed two additional hybridization signals which apparently represent duplication (-s) of the *hsp70-1* locus in *A. nanus*. The *A. nanus* isolates were further differentiated by the variable size of the first intron of the *hsp70-1* gene. This genomic variation in both neutral and under selection regions of the *hsp70* genes reflects an extensive genomic plasticity in *A. nanus*, which could be correlated with the plasticity in ecology and adaptation of this opportunistic nematode species.

Key words: *hsp70-1*, Southern hybridization, PCR, intron length polymorphism, gene birth and death.

Abbreviations: Hsp70, heat shock protein 70 kilodalton; ER, endoplasmic reticulum; MT, mitochondria; CYT, cytoplasm; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

INTRODUCTION

Seventy-kilodalton heat shock proteins (Hsp70s) function in a diverse set of biochemical processes, involving protein homeostasis and regulation of heat shock response (Boorstein *et al*., 1994). *Caenorhabditis elegans* and *C. briggsae* as well as all animal species encode several Hsp70s (Heschl & Baillie, 1990; Boorstein *et al*., 1994; Nikolaidis & Nei, 2004), which are targeted to endoplasmic reticulum (ER), mitochondria (MT) and cytoplasm (CYT). The CYT Hsp70 genes have followed a mixed evolutionary pattern with a combination of purifying selection and gene conversion-like events. This mode of evolution has resulted in different numbers of functional genes between the closely related nematodes *C. elegans* and *C. briggsae* and also between sibling species of *Drosophila* (Bettencourt & Feder, 2001; Nikolaidis & Nei, 2004).

Acrobeloides nanus (Cephalopidae) is a free-living, bacterial feeder, parthenogenetic nematode species, which shows differences from *C. elegans* during several stages of development (Wiegner & Schierenberg, 1998). *Acrobeloides nanus* is a dynamic colonizer (r-strategist), which exhibits high abundance and ecological plasticity, especially in extreme environments around the globe (Bongers,

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1990, 1999; Papatheodorou *et al*., 2004). Recently, different *A. nanus* populations and even different isolates were found to show high levels of genome plasticity, as exhibited by genomic divergence (intron length polymorphism) of the *hsp70-3* gene (Nikolaidis & Scouras, 2002).

The purpose of this study was to investigate the extent of genomic plasticity in *A. nanus* by examining the genomic divergence of a CYT Hsp70 gene among different isolates of the species. CYT *hsp70s*, due to their distinctive evolutionary pattern (see above), can be prominent molecular markers for the study of large-scale genomic events. In addition, the genomic differentiation of the *hsp70s* has been implicated in the adaptation and evolution of several organisms including nematodes (Cherkasova *et al*., 2000; Bettencourt & Feder, 2001; Michalak *et al*., 2001; Yokoyama *et al*., 2002).

MATERIALS AND METHODS

Nematode specimens

Nematodes were collected from Northern Greece (Petralona, Chalkidiki). All specimens reported here, were extracted from the same plot of soil (10 cm^3) and cultivated as single worm lines. Eleven out of the 36 nematode lines were identified as *A. nanus* using morphological characters. Nematode cultures and DNA extraction were performed according to Nikolaidis & Scouras (2002).

Southern hybridization

Three to four μg of genomic DNA were simultaneously digested with the restriction endonucleases *Bam*HI, *Eco*RI, *Sal*I (New England Biolabs), loaded onto a 1% agarose gel and transferred onto Hybond N^+ membrane (Amersham Pharmacia Biotech). The probe used was the *Eco*RI - *Sal*I fragment of *C. elegans hsp70-1* (2.4 kb), which contains the 5′-untranslated region including the promoter sequences and almost the complete coding region of this gene (Fig. 1a; clone pCes401; kindly provided by D. L. Baillie). Hybridization was performed at 37°C in Denhardt's solution containing 50% formamide, followed by medium stringency washes (3 × 15 min in 1 × SSC/0.5% SDS at 60°C), since the probe was heterologous. Plasmid DNA isolation, probe radiolabeling and autoradiography were performed as described by Konstantopoulou *et al*. (1998).

PCR amplification

The sequences of the primers used for the PCR amplification were 5′-CACCGAGCGTCTCATCG-3′ and 5′-CTCCGTGTCCCTCCTTG-3′ (forward and reverse, respectively) targeting the genomic sequen-

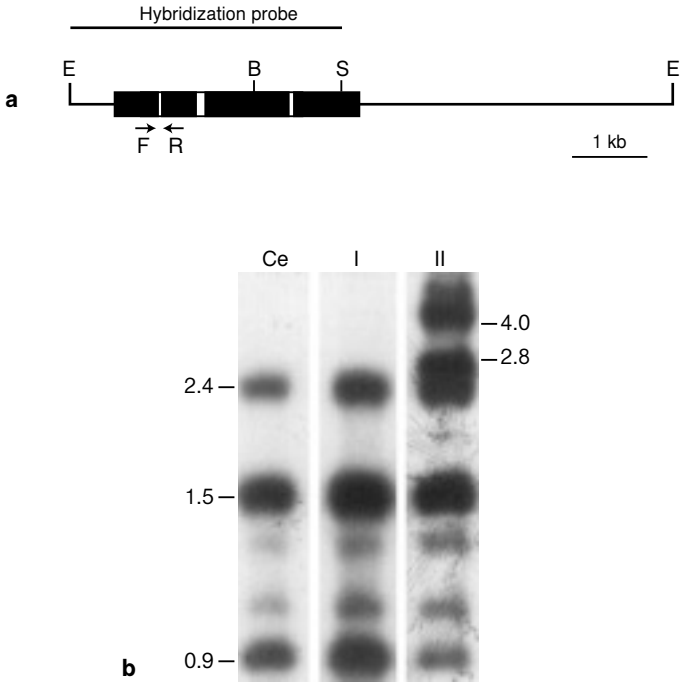

FIG. 1. (a) Genomic organization and restriction pattern of the *hsp70-1* gene in *Caenorhabditis elegans* (clone pCes401; Snutch *et al*. 1988). Black boxes represent exons and white boxes introns. Arrows indicate the PCR primers (F, forward; R, reverse; see text and Fig. 2 for nucleotide sequences). E, *Eco*RI; B, *Bam*HI; S, *Sal*I. (b) Different hybridization patterns observed among the *Acrobeloides nanus* isolates. The probe used was the *Eco*RI - *Sal*I fragment of *C. elegans hsp70-1* (2.4 kb). Hybridization was performed at 37°C in Denhardt's solution containing 50% formamide, followed by medium stringency washes. Lanes: Ce, *C. elegans*; I, *A. nanus* isolates of Pattern I; II, *A. nanus* isolates of Pattern II. Numbers indicate the genomic sizes in kilobases (kb).

ces that include the first intron of *hsp70-1* in *C. elegans* (Fig. 1a; Accession number M18540, F26D10.3). PCR amplification reactions were performed as in Nikolaidis & Scouras (2002), and the annealing temperature used was 48°C.

RESULTS

Southern hybridization

Two hybridization patterns of the *hsp70-1* probe were identified among the *A. nanus* isolates (Fig. 1b). Pattern I, which was observed in 9 out of the 11 isolates, presented three strong hybridization signals, of approximately 2.4, 1.5, and 0.9 kb size, and is similar to the respective one of *C. elegans* (Fig. 1b). Pattern II shares the 2.4, 1.5, and 0.9 kb bands with pattern I and presents two additional strong hybridization signals of about 2.8 and 4.0 kb size (Fig. 1b). The weaker hybridization signals observed in *C. elegans* and *A. nanus* could represent a cross-hybridization of the *hsp70-1* probe with other members of the Hsp70 multigene family, although their precise origin remains unclear.

The 2.4 kb band in *C. elegans* apparently represents the *Eco*RI - *Sal*I fragment (hybridization probe), while the 1.5 and 0.9 kb bands correspond to *Eco*RI - *Bam*HI, and *Bam* HI - *Sal*I sub-fragments of the 2.4 kb band (Fig. 1a, b; see also Snutch *et al*., 1988). Therefore, the 2.4, 1.5 and 0.9 kb bands in all *A. nanus* isolates should represent the orthologous *hsp70-1* gene in the latter species (Fig. 1b).

The high densities of the 2.8 and 4.0 kb hybridization signals in the *A. nanus* isolates of pattern II as well as the absence of other strong signals in *C. elegans* suggest that these additional bands also represent *hsp70-1* related sequences rather than cross-hybridization with other *hsp70s*. In addition, the 2.8 and 4.0 kb bands probably represent paralogous sequences of the *hsp70-1* gene in *A. nanus* rather than restriction fragment length polymorphism (RFLP). In the latter case, the isolates of pattern II should be heterozygous for one or more restriction sites of the *hsp70-1* gene, a hypothesis that could explain only one additional hybridization signal. Therefore, we suggest that at least one of the 2.8 and 4.0 kb bands represents a duplication of the *hsp70-1* locus in the *A. nanus* isolates of pattern II.

PCR amplification

After PCR amplification, four of the *A. nanus* isolates of pattern I presented a single band of approximately 500 bp, similar to that of *C. elegans* (Fig. 2). This band corresponds to the expected size of DNA fragments containing the first intron and its flanking sequences of the *hsp70-1* gene in the latter species (Fig. 1a). The remaining *A. nanus* isolates of pattern I and the two isolates that exhibited pattern II (Fig. 1b) presented a single band of almost 600 bp (Fig. 2). This polymorphism, which is apparently the result of variable intron length, differentiates further the *A. nanus* isolates. Genomic divergence, due to different intron lengths, has previously been reported for the *hsp70-3* gene in two *A. nanus* isolates (Nikolaidis & Scouras, 2002).

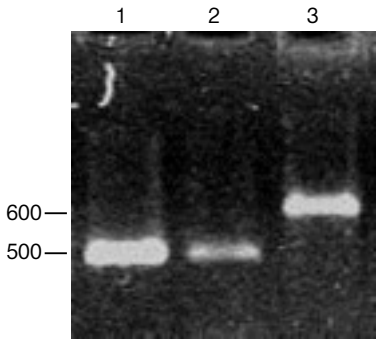

FIG. 2. PCR amplification products including the first intron of the *hsp70-1* gene for different *Acrobeloides nanus* isolates. The sequences of the primers used for the PCR amplification were 5′-CACCGAGCGTCTCATCG-3′ and 5′-CTCCGTGTCCCTCCTTG-3′ (forward and reverse, respectively) and the annealing temperature used was 48°C. Lanes: 1 = *C. elegans*, 2 = *A. nanus* isolates presenting the 500 bp band, 3 = *A. nanus* isolates presenting the 600 bp band. Numbers on the left indicate the PCR product sizes in base pairs (bp).

DISCUSSION

Our results show a substantial genetic differentiation associated with the *hsp70-1* gene among the *A. nanus* isolates, and also suggest that *hsp70-1* may be duplicated in two of the isolates. *Hsp70-1* in *C. elegans* is located in a highly divergent genomic region (Snutch & Baillie, 1984). This gene has been duplicated in *C. elegans* resulting in the truncated pseudogene *hsp-2* (reviewed by Heschl & Baillie, 1990). *Caenorhabditis briggsae* does not contain a sequence orthologous to the *C. elegans* pseudogene, as a result of the different rates of gene birth and death between the two closely related nematodes. The putative gene duplication in *A. nanus* suggests different rates of gene birth and gene death among isolates of the same

population. To our knowledge, this is the first time that such a case is described in the relevant literature for a metazoan species. Whether the paralogous sequences of *hsp70-1* in *A. nanus* are functional or represent pseudogenes, as in the case of *C. elegans*, requires further investigation.

Irrespective of the functionality of the additional *hsp70* copies, our results suggest extensive genome plasticity in *A. nanus*. Genome plasticity has been mainly reported for pathogenic bacteria and plants (Dorbrindt & Hacker, 2001; Ceccarelli *et al*., 2002). In these organisms genome plasticity is reflected by genomic alterations, frequent acquisition of DNA and loss of genetic information between domains that mainly contain repetitive DNA and allows rapid adaptation to environmental changes (Dorbrindt & Hacker, 2001; Rocha & Blanchard, 2002). Genomic divergence reflecting a substantial genomic plasticity has been described for microsatellite markers and the promoter of a highly heat-inducible Hsp70 gene between two *D. melanogaster* populations occupying different ecological niches (Michalak *et al*., 2001). *Acrobeloides nanus* represents a unique case, since different isolates from the same sampling plot exhibited both putative neutral (intron length) and under selection (gene number) genomic plasticity.

The genomic variation in Hsp70 genes could explain the extraordinary plasticity in ecology and adaptation, which allows species, such as *A. nanus*, to survive in a wide range of eco-geographical conditions. Further studies are essential to determine how genomic plasticity in the *hsp70s* is correlated with the ecological plasticity of the r-strategist nematode species *A. nanus*.

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