Mol Gen Genet (1984) 195:329–335

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A high degree of DNA strain polymorphism associated with the major heat shock gene in *Caenorhabditis elegans*

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Summary. We have searched for sequence variation between the Bristol and Bergerac strains of *C. elegans* in regions flanking three members of the 70 kilodalton (kd) heat shock peptide (hsp) gene family. No sequence variation was detected in 40 kb of DNA flanking two 70 kd hsp genes which are not stimulated by heat shock. In contrast, analysis of DNA flanking the heat shock inducible 70 kd hsp gene showed an unusually high amount of sequence variation between the two strains. Isolation and restriction map analysis of this gene from both strains revealed that the 5′ and 3′ flanking regions have diverged by 8.1 and 7.0% in nucleotide sequence, respectively. We have shown that these alterations are not due to large DNA rearrangements and conclude that the majority of sequence difference is the result of point mutations. Our results suggest that the heat shock inducible 70 kd hsp gene region accumulates mutations at a rate 10 to 20 fold higher than other regions of the genome. We propose that the anomalously high accumulation of mutational events is a direct consequence of the special status of the 70 kd hsp gene and its surrounding chromatin domain in the germline of *C. elegans*.

Introduction

In *Drosophila* and yeast the 70 kilodalton (kd) heat shock peptide (hsp) gene is a member of a small multigene family. Transcription of some of these members is inducible by heat shock, while transcription of others, termed cognates, occurs at normal temperatures and is not increased by heat shock (Ingolia and Craig 1982; Ingolia et al. 1982). To study the regulation and evolution of this interesting gene family we have cloned several members of the 70 kd hsp gene family from *C. elegans*. We have found in *C. elegans*, as has been found in *D. melanogaster* and *S. cerevisiae*, both heat inducible and non-heat inducible 70 kd hsp related genes (Snutch and Baillie, in preparation).

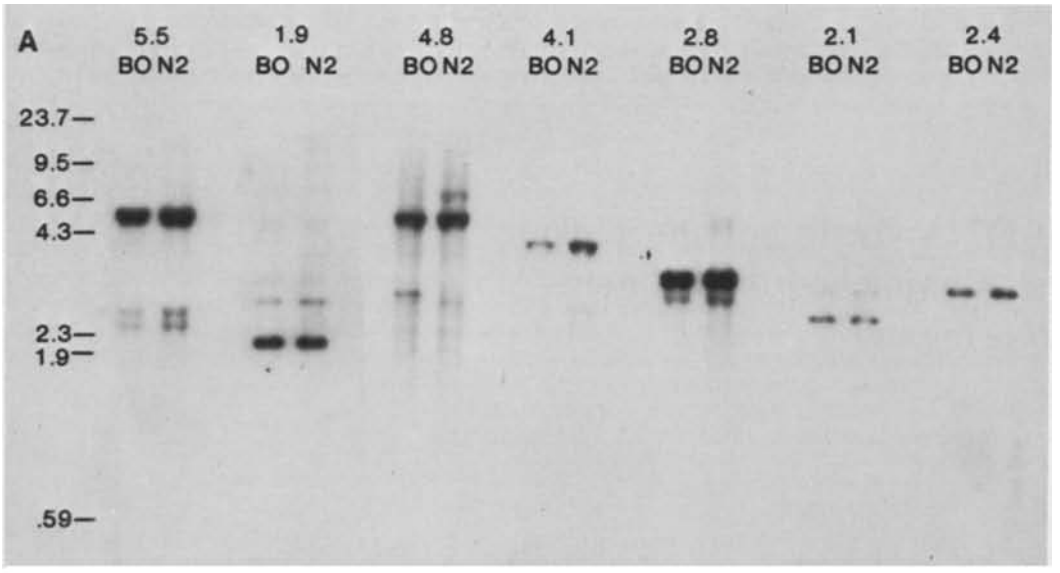
Analysis of a cloned gene is greatly facilitated if the genomic location of the gene and its neighbors are known. In order to map the cloned 70 kd hsp genes genetically we initiated a search for restriction fragment length differences (RFLDs) between two closely related strains of *C. elegans*. Previous studies into the sequence variation between the Bristol (N2) and Bergerac (BO) strains of *C. elegans* have shown the presence of a number of RFLDs (Emmons et al. 1979; Rose et al. 1982). For randomly cloned DNA fragments a divergence of about 1% in nucleotide sequence must be assumed in order to explain the observed frequency of RFLDs. The absence of detectable protein polymorphism between the N2 and B0 strains (Butler et al. 1981) suggests that the 1% sequence difference is distributed mainly in noncoding DNA regions. Using N2 and B0 interstrain crosses, the RFLDs can be mapped to chromosomal locations. This method involves a combination of standard genetic manipulations and hybridization to genomic DNA blots employing ^{32}P-labelled DNA fragments (Emmons et al. 1979; Rose et al. 1982).

In an attempt to identify RFLDs in regions surrounding the cloned 70 kd hsp genes we have found a region of the *C. elegans* genome in which RFLDs are highly clustered. Regions flanking the 70 kd hsp genes which are not transcriptionally activated by heat shock show a sequence divergence of approximately 0.5% between the two strains. In contrast, the regions flanking the heat shock inducible 70 kd hsp gene show a sequence divergence of about 10%. Our results suggest that some regions of the genome accumulate mutations at a rate 10–20 fold higher than other regions of the genome. We have shown, that unlike the RFLDs described previously (Emmons et al. 1983; Rose et al. 1982), these alterations are not the result of Tcl element transposition, nor of chromosomal rearrangements such as translocations, insertions, deletions or inversions. We propose therefore, that the increased accumulation of mutations results from the maintenance of the 70 kd hsp gene in a transcriptionally activatable configuration in the germline cells of *C. elegans*.

Materials and methods

Culturing. Caenorhabditis elegans var. Bristol, strain N2, and var. Bergerac, strain B0, were maintained on NGM plates as described by Brenner (1974). For RNA and DNA preparations *C. elegans* was cultured on high peptone plates seeded with a lawn of strain B. *Escherichia coli* as previously described by Rose et al. (1982).

Isolation of nucleic acids. For RNA preparations, worms were washed off plates with 0.04 M NaCl and pelleted lightly. RNA was subsequently isolated by the guanidine hydrochloride method described by Chirgwin et al. (1979) with modifications as described by Snutch and Baillie (1983). For genomic DNA preparations, worms were col-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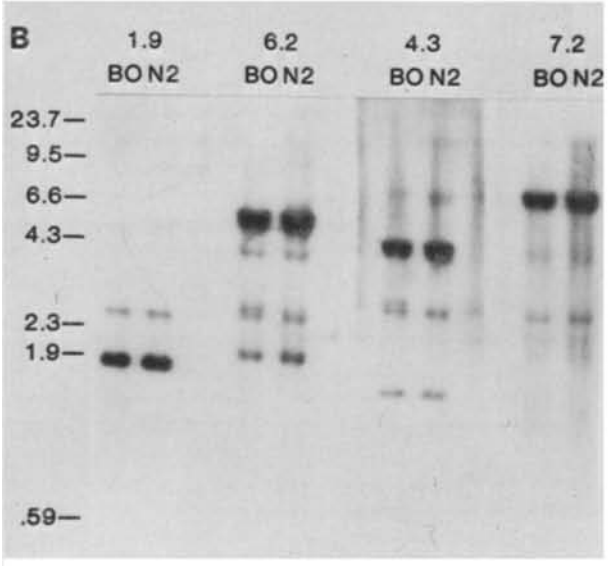
5.5 BO N2
1.9 BO N2
4.8 BO N2
4.1 BO N2
2.8 BO N2
2.1 BO N2
2.4 BO N2
23.7
9.5
6.6
4.3
2.3
1.9
.59


B
1.9 BO N2
6.2 BO N2
4.3 BO N2
7.2 BO N2
23.7
9.5
6.6
4.3
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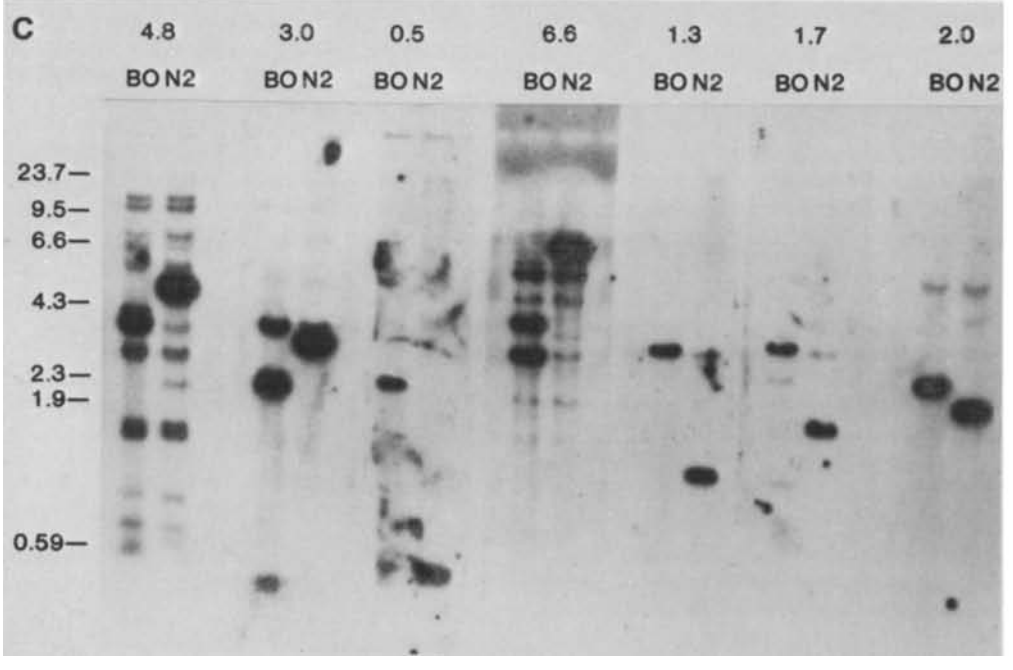
1.9
.59


C
4.8 BO N2
3.0 BO N2
0.5 BO N2
6.6 BO N2
1.3 BO N2
1.7 BO N2
2.0 BO N2
23.7
9.5
6.6
4.3
2.3
1.9
0.59

lected from plates with 0.04 M NaCl and gravity sedimented twice through this solution. Nematode DNA was isolated as described by Emmons et al. (1979), except that it was further purified through a 0.89 g CsCl, 0.25 mg ethidium bromide per millilitre equilibrium gradient.

Transfer of nucleic acids to nitrocellulose. DNA samples were digested with restriction endonucleases and 4 µg per lane was loaded onto agarose gels. Electrophoresis was performed as described by Snutch and Baillie (1983). DNA fragments were transferred to nitrocellulose by the bidirectional method of Smith and Summers (1980). Total RNA (17 µg per lane) was electrophoresed on a 1.1% agarose gel containing 2.2 M formaldehyde at 70 Volts for 5 h as described by Maniatis et al. (1982). After electrophoresis, the gel was soaked in water for 20 min, then transferred to nitrocellulose as described by Thomas (1980).

Hybridization. DNA and RNA filters were pretreated as described by Rose et al. (1982). DNA probes were nick-translated to a specific activity of approximately 10^7–10^8 cpm/µg using α-^{32}PdCTP and/or α-^{32}PdATP together as described by Rigby et al. (1977). Filters were washed and exposed to Kodak Blue Brand film with an intensifying screen at −80° C.

Lambda library construction. C. elegans var. Bergerac, strain B0, DNA was partially digested with EcoRI and electrophoresed through a 0.8% agarose gel. A gel slice containing DNA fragments between 14 and 22 kb was excised and the DNA electroeluted into dialysis tubing. Two µg of insert DNA was ligated to 0.5 µg of purified Charon 4 arms. Lambda packaging extracts and in vitro packaging were as described by Maniatis et al. (1982). The lambda library was screened according to Benton and Davis (1977). Phage were purified on a 0.75 g CsCl per millilitre equilibrium gradient. Phage DNA was purified as described by Davis et al. (1980). Construction of the N2 strain library will be described elsewhere (Snutch and Baillie, in preparation).

Subcloning. One µg of phage DNA was completely digested with EcoRI and ligated with 0.2 µg of the plasmid vector pUR2 (Rüther 1980). Positives were selected on ampicillin, XGAL, IPTG plates. Mini-DNA plasmid preparrations were according to Davis et al. (1980). Large scale plasmid preparations were in minimal media as described by Maniatis et al. (1982).

Calculation of sequence divergence. For each strain the total number of hexanucleotide restriction sites times six was divided into the number of restriction sites different in the other strain. This was calculated for each strain and the average was taken. (see Bender et al. 1983).

Results

Using DNA from the N2 strain of *C. elegans* we have constructed on EcoRI partial digest library in the lambda vector, Charon 4. Screening this library with a probe encoding the *Drosophila* 70 kd hsp, we isolated three distinct classes of lambda clones, each representing a single gene. One gene, A, showed strong homology to the *Drosophila* probe and has been shown to be heat inducible. The other two B, and C, represent genes which were somewhat less homologous to the *Drosophila* gene. Transcription of the class C gene was not increased by heat shock; similar to the 70 kd hsp cognate genes of *Drosophila* and yeast described by Ingolia et al. (1982). Transcription of the class B gene has not been detected (Snutch and Baillie, in preparation).

In order to map the 70 kd hsp related genes, suitable probes for RFLDs were required. Each class of lambda phage was digested with EcoRI and subcloned into the plasmid vector pUR2. From the class A lambda clones, seven subclones representing 19 kilobases (kb) of DNA were derived. Similarly, seven subclones from the class B and four from the class C, representing 24 and 20 kb, respectively, were obtained. In total, 63 kb of DNA representing about 0.1% of the *C. elegans* genome were subcloned for use as specific hybridization probes.

Figure 1 shows the results of hybridization of the class A, B and C region probes to EcoRI digests of N2 and B0 strains DNA. For the class B and C genes, comparison of the N2 and B0 genomes for RFLDs shows that flanking regions are identical for EcoRI sites. In fact, digestion with six other restriction enzymes and hybridization

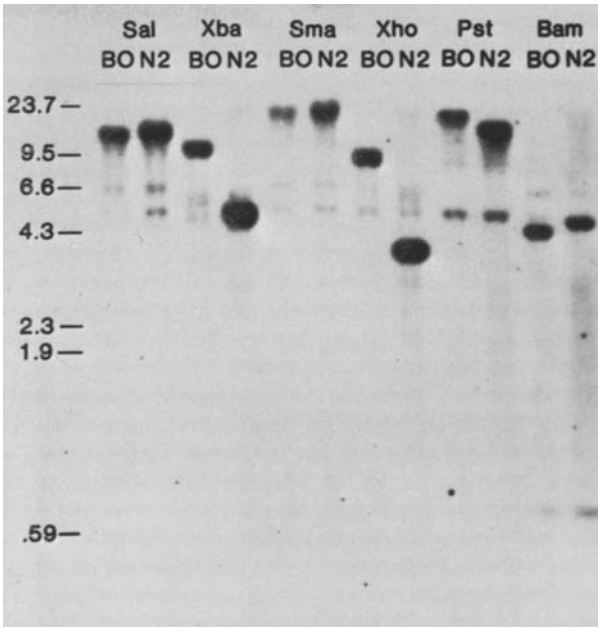


Fig. 2. Hybridization of the 3.0 kb class A sublcone to N2 and B0 DNA digested with various restriction enzymes. N2 and B0 DNA was cut with either SalI, XbaI, SmaI, XhoI, PstI or BamHI. Four of the six digests show clear RFLDs. Markers are HindIII digested lambda DNA

Fig. 1a–c. Hybridization of subcloned fragments derived from regions flanking three cloned 70 kd hsp genes to N2 and B0 strain DNA. Probes are EcoRI subclones of phage isolated from an N2 strain genomic library constructed in Charon 4. **a** EcoRI subclones from the class B 70 kd hsp gene. The 4.8 kb fragment contains the coding element; **b** Subclones from the class C 70 kd hsp gene. The 6.2 and 4.3 kb fragments contain the coding element; **c** Subclones from the class A 70 kd hsp gene. The 6.6 kb fragment contains the coding element. None of the class B and C subclones shows any EcoRI site RFLDs. All seven class A subclones show marked RFLDs. Markers are HindIII digested lambda DNA

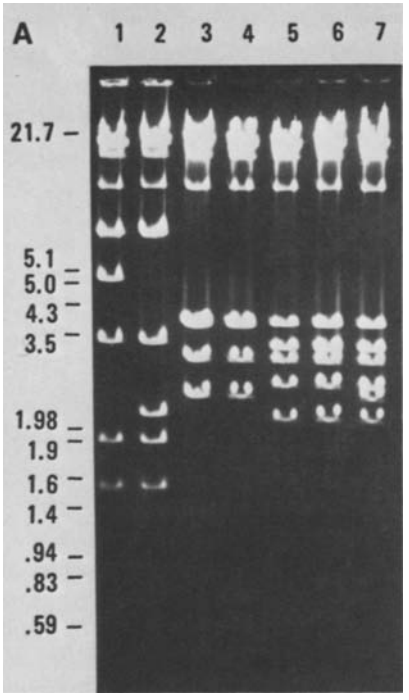
with several probes flanking these genes indicates that regions flanking B and C are conserved between the two strains (Snutch, unpublished results).

In contrast, comparison of the class A clones shows a surprising result: every EcoRI subclone shows a RFLD between the two strains. The nature of the RFLD varies from fragment to fragment. Some N2 strain EcoRI fragments shift to larger fragments (for example, the 2.0, 1.7, 1.3, and 0.5 kb), while others are split into two or more fragments in the B0 strain (for example, the 6.6, 3.0 and 4.8 kb). Examination of this region with other restriction enzymes shows that this high frequency of RFLDs is not limited to EcoRI sites. Figure 2 shows the hybridization of the class A 3.0 kb probe to N2 and B0 DNA digested with several restriction enzymes. Four of the six enzymes tested show RFLDs. From these results, we can estimate the minimum amount of sequence divergence flanking the class B and C genes to be less than 0.5%, whereas that flanking the class A gene to be greater than 10%.

We believe that this elevated frequency of divergence reflects a higher mutational sensitivity in this region. It is possible that the special status of the 70 kd hsp gene, which must be transcriptionally accessible at all times, is reflected in the structural conformation of its chromosomal domain.

The 70 kd hsp gene is known to be inducible in all cells which are transcriptionally competent (Dura 1981; Bensaude et al. 1983). Thus, it is reasonable to assume that this gene may lie in an accessible conformation even in the germline. The accessibility of this domain could also apply to mutagenic agents. Since it is germline tissue, spontaneous mutations would therefore be inheritable and could, after some generations, have accumulated to the high number we observe. This scenario would not be true for genes in an inaccessible or protected conformation in the germline (the hsp cognate genes for example).

Several alternate explanations have been eliminated. First, the possibility that DNA rearrangements have occurred has been tested by examination of cloned DNA from the B0 strain. An EcoRI partial digest library of the B0 strain was constructed in Charon 4. Screening of this library with the N2 class A 6.6 kb subclone produced five overlapping clones. Figure 3a shows the ethidium bromide stained pattern of the N2 and B0 class A phage. It is evident that none of the EcoRI fragments are the same size between the two strains. Hybridization of the individual N2 subclones to blots of the B0 phage indicates that while EcoRI RFLDs have occurred between the two strains, the majority



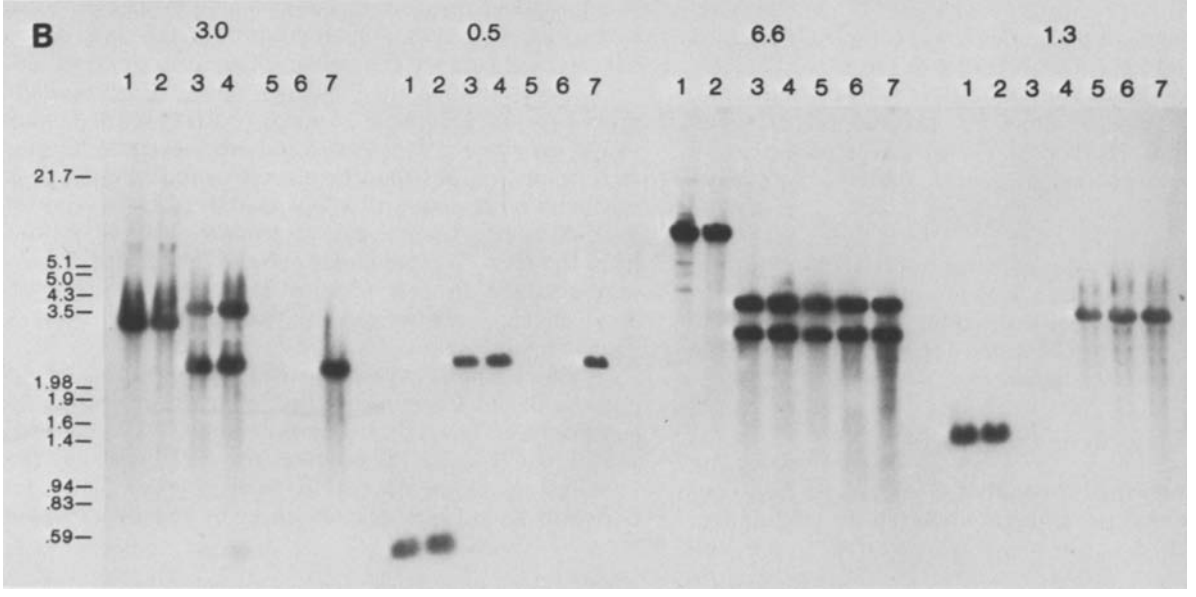


Fig. 3a, b. Comparison of the class A 70 kd hsp gene of the N2 and B0 strains. **a** Ethidium bromide stained gel of two N2 strain class A phage (lanes 1 and 2) and five B0 strain clones of the class A region (lanes 3–7). **b** Autoradiogram of the above gel after hybridization to the 3.0, 0.5, 6.6 and 1.3 kb class A subclone. Due to the overlapping nature of the lambda phage, only the 6.6 kb probe hybridizes to all seven clones. Markers are EcoRI/HindIII digested lambda DNA

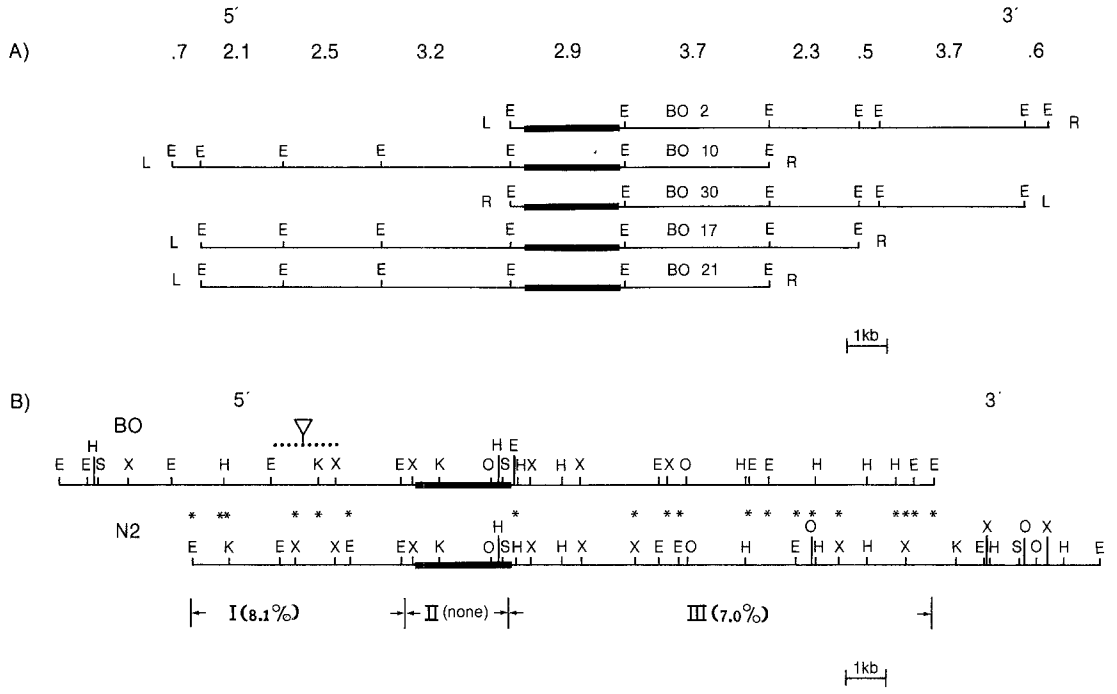


Fig. 4a–b. Restriction map of the N2 and B0 class A heat shock genes. **a** Linear arrangement of the overlapping B0 phage. **b** Comparison of the restriction maps of the N2 and B0 class A gene region. Asterisks mark sites of an RFLD caused by either generation or loss of a restriction site. Inverted triangle 5′ to the coding element represents site of a 200 bp insertion. *H*, HindIII; *E*, EcoRI; *X*, XbaI; *O*, XhoI; *S*, SalI; *K*, kpnI

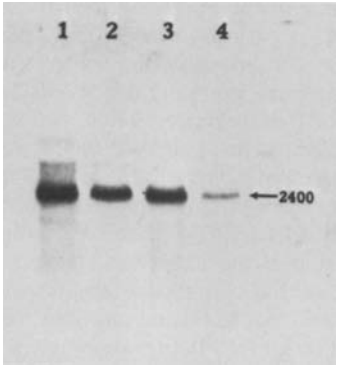


Fig. 5. Hybridization of the N2 class A gene to N2 and B0 RNA. Lanes 1 and 2 are N2 heat shock and control RNA, respectively. Lanes 3 and 4 are B0 heat shock and control RNA. Sizes are in nucleotides

of sequences remains the same (Fig. 3b). The RFLDs present in the lambda phage are identical to those occurring in the genomic blots (Fig. 1c and Fig. 3b). Furthermore, the linear arrangement of EcoRI sites in the N2 and B0 phage shows the class A heat shock regions of these two strains to be colinear.

We have restriction mapped the B0 class A region and compared it to the N2 class A region (Fig. 4). The results show that there is an overall sequence divergence of about 6.0% over the 20 kb of DNA compared. However, the sequence divergence is not equally distributed throughout the region. Figure 4b shows that 5 kb of DNA flanking the gene 5′ and 12 kb flanking 3′ have diverged by 8.1% and 7.0%, respectively, between the two strains. In contrast, no sequence difference has been detected within the 70 kd hsp coding element. The nearest detected sequence alteration is the introduction of an EcoRI site about 150 bp 3′ to the coding region in the B0 strain. The nearest detected alteration in the 5′ flanking region is the loss of an EcoRI site about 1.5 kb from the coding element.

Comparison of the flanking regions shows that about 53% of the hexanucleotide restriction sites are identical between the two strains. The colinearity of the overall region indicates that most of the restriction site changes are not the result of large DNA rearrangements. In fact, the only rearrangement detected is the insertion of about 200 bp of DNA between 1.5 and 3.0 kb 5′ to the coding element (we would not have detected deletions and insertions less than 100 bp).

Second, *C. elegans*, like many organisms, contains mobile genetic elements, thus, it is possible that RFLDs result from transposon insertion or deletion events (Emmons et al. 1983). The N2 and B0 strains contain approximately 25 and 250 copies, respectively, of the transposon, Tcl. Hybridization of a Tcl element (thought to be the only *C. elegans* transposon, Emmons et al. 1983) shows that neither strain carries this element in the class A 70 kd hsp region (Snutch, unpublished results).

Third, the B0 class A gene is not a pseudogene. Since, our previous results indicate that *C. elegans* has only one class A heat shock inducible 70 kd hsp gene, we tested the heat shock response of the B0 strain. At elevated temperatures the N2 and B0 strains synthesize similar amounts of the 70 kd hsp in vivo, indicating that the B0 strain has an active, inducible 70 kd hsp gene (Snutch, unpublished results). Fig. 5 shows the results of hybridization of the class A gene to heat shock and control RNA from N2 and B0 worms. Both strains produce a heat inducible message of 2,400 nucleotides in size. We conclude that the B0 class A gene is actively transcribed in response to heat shock.

Fourth, it is possible that the high frequency of RFLDs is the result of a feature of the chromosomal location of the class A heat shock gene. It has been genetically mapped to the far right end of chromosome IV (Snutch and Baillie, in preparation). Other, randomly selected RFLDs, have previously been shown to map at many locations in the *C. elegans* genome, and not specifically to chromosome ends. Thus, we feel the chromosomal location of the class A gene does not account for the elevated RFLD frequency. Therefore, we are left with the possibility that the structural conformation of the 70 kd hsp gene's chromatin domain in the germline of *C. elegans* has led to an accumulation of mutational events, resulting in the high level of sequence divergence observed in this study.

Discussion

We have compared the sequence variation between two strains of *C. elegans* for three cloned regions of the genome. Unexpectedly, the DNA flanking the inducible 70 kd hsp gene shows a sequence divergence of about 7.5%. This region appears to have accumulated an unusually high number of point mutations in addition to at least one insertion event. Comparable sequence differences have been described for organisms which are separated for millions of years (for example, cow and sheep, 11.2%, Kohe et al. 1972; mouse and rat, 9%, McCarthy and Farquhar, 1972).

We have attempted to rule out several possible mechanisms for the occurrence of this high sequence divergence. Isolation of the corresponding class A 70 kd hsp gene from a Bergerac genomic library and hybridization of Bristol DNA flanking probes shows that the 70 kd hsp coding element is surrounded by essentially the same DNA in both strains. This indicates that no localized DNA rearrangements have occurred. In addition, we have mapped the 70 kd hsp gene to chromosome IV in both strains, indicating no major DNA rearrangements have occurred (Snutch, unpublished results). Leigh Brown (1983) has shown a high frequency of insertion/deletion events has occurred 5′ to the coding elements at the 87 A heat shock locus in natural populations of *Drosophila melanogaster*. One class of insertion elements consists of 100–200 bp fragments. The second class is comprised of known transposable elements. In addition to the many apparent point mutations flanking the Bristol and Bergerac 70 kd hsp coding element, we have found the insertion of a 200 bp fragment 5′ to the gene. There are no copies of the *C. elegans* transposon, Tc1, in the 20 kb of flanking DNA in either strain.

In the *D. melanogaster* Oregon R and Canton S strains a nucleotide difference of about 0.4% exists (Bender et al. 1983). Two previous studies have used randomly cloned DNA fragments to analyse the sequence divergence between the Bristol and Bergerac strains of *C. elegans* (Emmons et al. 1979; Rose et al. 1982). These studies concluded that a nucleotide difference of about 1% exists. Emmons et al. 1983) have previously noted that this difference is rather high for two closely related strains but that most of the 1% divergence can be accounted for by the high number of Tc1 insertions in the Bergerac strain. Our results suggest that the reported 1% sequence difference between the Bristol and Bergerac strains of *C. elegans* varies by as much as ten fold in some regions of the genome. In some regions of the genome no divergence occurs. For example, other researchers have not detected any RFLDs in large regions of the *C. elegans* genome flanking three myosin genes (G. Benian and R. Waterston, pers. comm.).

Two recent studies on *D. melanogaster* have analysed the alcohol dehydrogenase (Kreitman 1983) and the 87A heat shock locus (Leigh Brown 1983) for sequence variation in a large number of individual chromosomes. Both have concluded that different regions of the genome may be subject to different mutation rates or selective constraints. We propose that the increased frequency of RFLDs around the *C. elegans* class A kd hsp gene is a reflection of its unique chromatin domain in gonadal tissue.

It is generally thought that eukaryotic DNA exists as a 200–300 A chromatin fibre which is folded into looped domains (for review see Chambon 1978). Evidence suggests that each looped domain, containing between 45 and 90 kb of DNA (Benyajati and Worcel 1976; Pinon and Salts 1977), may correspond to individual transcription units (Korge 1977; Lamb and Daneholt 1979). It has also been shown by electron microscopic and biochemical analysis, that genes which are active or potentially active have altered properties suggesting that these chromatin domains exist in a decondensed, extended form (Weintraub and Groudine 1976; Garel and Axel 1976; Howard et al. 1981; Andersson et al. 1980; Foe 1978). Transcription of the 70 kd hsp gene has been shown to be heat inducible in a large number of species (Tissieres et al. 1974; Miller et al. 1979; Kelly and Schlesinger 1978; Snutch and Baillie 1983; Loomis and Wheeler 1980). It is found in all adult tissues tested, including significantly the gonad (Lewis et al. 1975; Zimmerman et al. 1983; Currie and White 1983). It would therefore be expected that this particular chromatin domain is in an active or potentially active configuration at all times. We propose that maintenance of the active configuration or actual transcriptional activity in the germline tissue will be conducive to increased mutational events which can be passed onto the next generation. If this proposal is correct we would predict that the increased accumulation of mutations would cease abruptly at the boundaries of the hsp 70 domain. We have recently successfully cloned flanking DNA 20 to 30 kb on either side of the 70 kd hsp gene and as predicted the occurrence of RFLDs falls dramatically in the limits of this region (K. Beckenbach, unpublished results). It will be interesting to know whether other germline expressed genes show a similar phenomenon.

Acknowledgements. We thank K. Beckenbach for technical assistance, I. Kovesdi for Charon 4 DNA and Dr. A. Rose for the Tc1 element (HindIII variant). We are grateful to Drs. B. Honda, J. Curran, A. Rose and M. Smith for their comments and discussion. This work was supported by the Natural Sciences and Engineering Research Council, the Muscular Dystrophy Association and the National Cancer Institute of Canada. T.P.S. is supported by an S.F.U. Open Graduate Scholarship and a NSERC postgraduate scholarship. The B0 strain of *C. elegans* was obtained from the *Caenorhabditis* Genetics Gentre which is supported by contract number NO1-AG-9-2113 between the Curators of the University of Missouri and NIH.

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Communicated by E. Bautz

Received November 29, 1983