The heat shock 70 gene family in the Mediterranean fruit fly *Ceratitis capitata*

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**Abstract**

The cloning and the characterization of the heat shock 70 (hsp70) genes of the medfly *C. capitata*, a major agricultural pest, are presented. Six genomic clones were isolated by screening a medfly genomic library with an hsp70 genomic fragment of *Drosophila melanogaster*. They form two 30 kb contigs, both of which map cytogenetically in a single major heat shock puff (3L:24C) of the salivary gland polytene chromosomes. Restriction mapping and blot hybridization indicated the presence of six putative hsp70 genes in these two closely linked regions. The sequence of one of these genes suggests that it is a heat-inducible hsp70 gene. The 638-codon open reading frame shows 84% identity at the amino acid level (73.5% at the nucleotide level), relative to corresponding *D. melanogaster* sequences. The 5' untranslated leader sequence, approximately 200 bp long, is not interrupted by introns and is very rich (48%) in adenine residues, resembling *Drosophila* heat-inducible hsp70 genes. Furthermore, the promoter of this gene contains two characteristic heat shock elements close upstream from the TATA box. The levels of the hsp70 transcripts are very low at 25–30°C, increase significantly at 33°C and reach maximum at 39°C.

**Keywords:** hsp70 genes, *Ceratitis capitata*, heat shock.

**Introduction**

The genes coding for the heat shock 70 proteins (hsp70 genes) have been studied in many organisms. In eucaryotes they are members of a multigene family and are highly conserved, especially in the N-terminal coding region which corresponds to an ATPase domain (Lindquist & Craig, 1988; Feige & Polla, 1994, for reviews). Both structure and regulation of the hsp70 genes have been studied extensively. The DNA sequences responsible for regulating heat shock gene expression in eukaryotes are known as heat shock elements (HSE) and are invariant from yeast to human (Schlesinger, 1990). The HSEs consist of variable numbers of the 5 bp sequence nGAAn, arranged in alternating orientation. As has been shown in *Drosophila*, a functional HSE contains a minimum of three contiguous nGAAn units and two HSEs, positioned close to the transcriptional start site, are sufficient for maximum heat shock induction (Amin et al., 1988; Simon & Lis, 1987). The hsp70 genes studied so far share several common features. The genes lack introns (Lindquist & Craig, 1988) and their mRNAs have long 5'-untranslated leader sequences unusually rich in adenine residues, conferring preferential translation, and AU regions in the 3'-untranslated sequence, providing differential stability under normal and heat shock conditions (Lindquist & Petersen, 1990, for a review).

The medfly, *Ceratitis capitata* (family Tephritidae), is a widespread and destructive insect pest of soft fruit (Christenson & Foote, 1960). Genetic engineering strategies are expected to improve existing biological control methods, such as the Sterile Insect Technique (Louis et al., 1987). *C. capitata* has well-characterized polytene chromosomes which facilitate cytogenetic analysis (Beda, 1987; Zacharopoulou, 1990; Zacharopoulou et al., 1992) and a recently developed germline transformation system that allows genetic engineering in this species (Loukeris et al., 1995). An important component of a gene expression system is the promoter used to express the gene of interest. The *D. melanogaster* hsp70 promoter has been a popular choice for expression of genes in other insect species (Berger et al., 1985; Miller et al., 1987; O’Brochta & Handler, 1988; Atkinson & O’Brochta, 1992; Loukeris et al., 1995); however, the strength of this promoter in non-drosophilid systems has been reported to be low.
(Berger et al., 1985; Atkinson & O’Brochta, 1992). Therefore, it seems that if high levels of conditional gene expression are required, then a homologous hsp70 promoter should be considered. By analogy with D. melanogaster, gene transfer vectors containing medfly hsp70 promoters would be a valuable molecular genetic tool, enabling widespread, efficient and easily regulated expression of engineered gene constructs in this insect pest. As a first step towards this goal, we report here the isolation of the medfly hsp70 genes. The genomic organization of two clusters of medfly hsp70 genes is described and the complete sequence of one of these genes is reported.

**Results**

**Cloning and organization of C. capitata hsp70 genes**

Figure 1 shows the results of a Southern hybridization experiment in which two DNA fragments from the 5’ and 3’ ends, respectively, of the coding region of a D. melanogaster hsp70 gene were used to probe medfly genomic DNA, digested with Hind III, under relatively high stringency conditions (see Experimental procedures). D. melanogaster genomic DNA was also analyzed in the same blot. Both probes gave five major bands (18–4.4 kb) and three minor ones (3.2–1.2 kb) with medfly DNA, suggesting the presence of multiple hsp70 genes in this genome. The same probes hybridized strongly with two large Hind III fragments of the D. melanogaster DNA (10–14 kb), corresponding to the five hsp70 genes identified in this species (Mirault et al., 1979).

To clone the hsp70-related sequences from medfly, a genomic λ EMBL4 medfly library (Rina & Savakis, 1991) was used. Approximately $3 \times 10^6$ recombinant phage plaques were screened with the D. melanogaster hsp70 5’-probe. Six positive clones with strong signals were purified to homogeneity and characterized by a combination of restriction mapping and Southern blot analysis. The results obtained are summarized in Fig. 2. The six clones are organized in two regions (A, B), each covering approximately 30 kb. Specific probes from the A and B regions hybridized to the same band of the polytene chromosomes suggesting that the two regions are closely linked (see below). Southern blot analysis revealed that the two cloned regions contain six putative hsp70 genes (A1, A2, A3, B1, B2, B3) separated by sequences of 3.5–8 kb, as shown by boxes in Fig. 2. All restriction fragments of the regions marked with boxes hybridized either to one or both 5’ and 3’ Drosophila hsp70 probes, as well as to each other (results not shown). These results are in agreement with those obtained from the genomic southern analysis (Fig. 1). As shown in Fig. 1, the sizes of the five major Hind III bands are 4.4, 5, 6, 11 and 18 kb. The 4.4 kb band matches with the Hind III fragments of the A3 and B2 genes, the 5 kb band matches with the Hind III fragment of the B1 gene and the 6 kb band matches with the Hind III fragment of the A2 gene. The other two bands (11 and 18 kb) most likely correspond to A1 and B3 genes. The above data suggest the presence of six hsp70 genes per haploid genome in medfly. Furthermore, the 5 kb band has similar intensity to the 4.4 kb band which represents two genes (A3 and B2), suggesting that a seventh unidentified gene may also exist. In order to find out whether both gene clusters are present in single animals, Hind III restricted DNA samples from single adults were probed with two specific a and b probes indicated by bars in Fig. 2. As is shown in Fig. 3, all eight individuals tested gave the same bands with the expected sizes (4 and 3.8 kb), indicating that both clusters exist in the medfly genome. Based on the differential hybridization of the restriction fragments to the 5’ and 3’ Drosophila hsp70 probes, we were able to determine the orientation of four of these genes as indicated by the thick arrows under the boxes in Fig. 2. The detailed restriction map of one of these genes and
its immediate 5' flanking region is shown (Fig. 2C). We call this gene Cchsp70-B1.

**Structure of the Cchsp70-B1 gene and conserved features between C. capitata and D. melanogaster**

Sequencing as indicated by the thin arrows in Fig. 2C showed that this region contains a gene highly homologous to *D. melanogaster* and other eukaryotic hsp70 genes. The nucleotide sequence and the deduced amino acid sequence of this gene are shown in Fig. 4. The Cchsp70-B1 gene has a single open reading frame of 1914 bp that potentially encodes a 638 amino acid polypeptide with an estimated molecular weight of 70.07 kDa. The coding region starts with an ATG at base 679 of the sequence and ends at position 2592. The nucleotide sequence surrounding the initiation codon (CAAAATG) is in good agreement with the consensus sequence C/AAAA/CATG flanking translational start sites in *Drosophila* (Cavener, 1987). A TATA box centred at position 450 was found 30 bp upstream from a putative cap signal (TCAGAATT) centred at position 480. It must be noted here that putative cap signals are also present in the heat-inducible hsp70 genes of *D. melanogaster*, although their mRNAs do not seem to require cap-binding factor for efficient initiation of translation (Linquist & Petersen, 1990; for a review). Splice junction features were not present in the region between the putative cap signal and the initiation codon of the Cchsp70-B1 gene, indicating that the medfly gene has a long (approx. 200 bp) 5'-untranslated region, not interrupted by introns. This region was very rich in adenine residues (48%).

Upstream and close to the TATA box, two characteristic heat shock regulatory elements (HSEs) were identified, centred at positions 423 and 405. Each of them consists of three contiguous nGAAn units, arranged in alternating orientation. In the HSE closer to the TATA box, one of the units is variant (nGAGn). This sequence is the most frequently found variant in natural HSEs (Amin et al., 1988).

We compared the sequence of the Cchsp70-B1 gene with two heat-inducible hsp70 genes of *D. melanogaster* (DMHSP7D1 and DMHSP7A2; Ingolia et al., 1980). The comparison showed a high degree of con-
servation in the coding region at both nucleotide level (73.3\% and 73.5\% respectively) and amino acid level (84\%). The similarity in the 5’ and 3’ untranslated regions (UTRs) was much lower although two features of the Drosophila genes, high percentage of A in the 5’ UTR and AT rich sequences in the 3’ UTR, were conserved. Figure 5 shows an alignment of the deduced amino acid sequences of the medfly and Drosophila genes. A striking degree of conservation was found in the N-terminal region extending to position 520 of the medfly protein whereas the conservation in the C-terminal region was less pronounced. In sixteen of the eighteen positions in which the D. melanogaster proteins have different amino acids, indicated by boxes, the medfly protein has the same amino acid with either one or the other Drosophila protein. Table 1 shows an amino acid identity matrix which includes hsp70 proteins from different organisms and an hsp70 cognate of medfly. The Cchsp70-B1 protein is more similar to D. melanogaster proteins (84\%) and less similar to Xenopus and human proteins (72.7\%). The similarity to the medfly cognate protein (73.5\%) was found to be much lower than the similarity between medfly hsp70 and other insect hsps. Table 2 shows a comparison of the codon usage between medfly and Drosophila hsp70 genes. Both D. melanogaster genes exhibit a deficiency of A and T in the third position of the codons which is typical for most abun-

Figure 3. Southern hybridization of restricted genomic DNA from single flies. DNAs from single flies were digested with Hind III, separated on 1\% agarose gels, and blotted on a Nytran-plus membrane filter. The blots were hybridized at high-stringency conditions (6 \(\times\) SSC, 70°C) with the 4 kb Hind III fragment of \(\lambda\)D6 clone (A) and with the 3.8 kb Hind III fragment of \(\lambda\)D3 clone (B), indicated by the dashed lines in Fig. 2.

<table>
<thead>
<tr>
<th></th>
<th>Cchsp70-B1</th>
<th>D. mel.</th>
<th>D. aur.</th>
<th>A. alb.</th>
<th>C. elegans</th>
<th>Xenopus</th>
<th>Human</th>
<th>Cchsc70</th>
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<td>0.735</td>
</tr>
<tr>
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<td>0.947</td>
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<tr>
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<td>1.000</td>
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Figure 4. Complete nucleotide sequence of the Cchsp70-B1 gene (EMBL database accession no. Y08955). The predicted amino acid sequence is shown below the DNA sequence. The features of the nucleotide sequence that are discussed in the text have been underlined. These features from 5' to 3' are: two putative heat shock elements, TATA box, a putative cap signal, translation initiator and termination codon. Numbers refer to nucleotide and amino acid positions.

Figure 4 (continued).

**Figure 5.** Comparison of the deduced *C. capitata* hsp70 amino acid sequence to the amino acid sequences of two *D. melanogaster* hsp70 genes (DMHSP7A2, GenBank accession nos. J01103 and DMHSP7D1, GenBank accession no. J01104). Asterisks indicate perfectly conserved positions and dots well-conserved positions among the three sequences. Boxes indicate identity between *C. capitata* protein and either one or the other *D. melanogaster* proteins.

dantly expressed genes of this species (Shields et al., 1988). In contrast, in the medfly gene the GC content in the third position of the codons is similar to the total GC content (see Discussion).

Concentration of hsp70 transcripts during heat shock and recovery

Equal amounts of total RNA, from third-instar larvae incubated for 30 min at various temperatures, was analysed by Northern hybridization. The coding region of the Cchsp70-B1 gene was used as a probe (Fig. 6A). One band of 2.4 kb, consistent with a RNA molecule encoding a protein of 70 kDa, was obtained. The levels of the hsp70 transcripts were found very low at temperatures up to 30°C. Maximum induction was obtained at 39°C, whereas the induction at 42°C was found similar to that at 36°C. At higher temperatures the survival of the larvae was limited. Figure 6B shows the levels of the hsp70 transcripts during recovery from a heat shock. Third-instar larvae, incubated for 30 min at 39°C, were transferred to 25°C for various time periods and their RNA was analysed as above. The concentration of the hsp70 transcripts increases during the first 15 min and then decreases gradually, reaching

the non-heat shock levels within 90 min from the transfer to 25°C. These data are similar to those reported for D. melanogaster and A. albimanus (Di Domenico et al., 1982; Benedict et al., 1993), suggesting degradation of the hsp70 transcripts during recovery as has been shown in D. melanogaster (Lindquist & Petersen, 1990, for a review).

Chromosomal localization of the medfly hsp70 genes

In order to find the chromosomal localization of the Cchsp70-B1 gene, we performed in situ hybridizations on salivary gland polytene chromosomes from untreated (25°C) and heat shocked (39°C for 30 min) 5-day-old larvae using as a probe a DNA fragment containing the coding region of the Cchsp70-B1 gene. The results are shown in Fig. 7. A single hybridization signal was found in the band 24C on the left arm of chromosome 3 (Zacharopoulou, 1990), as shown in Figs 7A and 7B. This band corresponds to one of the major heat shock puffs, as shown in Figs 7C and 7D. The same in situ hybridization experiments were performed with all six clones and the two specific a and b probes of the two gene clusters (Fig. 2). All probes gave a single hybridization signal in the band 24C, suggesting that the medfly hsp70 genes are closely linked (results not shown).

Discussion

The data presented here suggest that we have cloned six medfly hsp70 genes which are distributed in two 30 kb units. The existence of an hsp70 multigene family in the medfly is in agreement with what is known for several other eukaryotic species (Lindquist & Craig, 1988; Gunther & Walter, 1994, for reviews). All medfly hsp70 genes were mapped to the same polytene chromosome band (3L:24C), corresponding to one of the major heat shock puffs. This implies that the genes are closely linked and heat-inducible. The chromosomal arrangement of the medfly hsp70 genes differs from the D. melanogaster and Anopheles albimanus organization, where the hsp70 genes are mapped at two discrete loci (Ish-Horowicz & Pinchin, 1980; Benedict et al., 1993). Other Drosophila species such as D. repleta (Peters et al., 1980), D. obscura (Molto et al., 1992) and D. montium (Drosopoulou et al., 1996) have a single hsp70 locus. A single hsp70 locus also exists in other Terphritidae species such as Bactrocera tryoni (unpublished results) and Bactrocera oleae (Mavrayani-Tsipidou, personal communication). Furthermore, the structure of the medfly genes within each unit is different from that of D. melanogaster genes. In D. melanogaster the genes of each unit are tightly linked and have identical restriction patterns (Mirault

Table 2. Comparisons of GC content between medfly and D. melanogaster hsp70 genes. (G + C) \text{\%}: total GC content in the coding sequences. (G + C)_{III}\%: Total GC content at position III of codons.

<table>
<thead>
<tr>
<th>Gene</th>
<th>(G + C)%</th>
<th>(G + C)_{III}%</th>
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</thead>
<tbody>
<tr>
<td>DMHSP7D1</td>
<td>57.2</td>
<td>73.8</td>
</tr>
<tr>
<td>DMHSP7A2</td>
<td>58.4</td>
<td>75.5</td>
</tr>
<tr>
<td>Cchsp70-B1</td>
<td>49.4</td>
<td>53.3</td>
</tr>
</tbody>
</table>

Figure 6. Northern analysis of the hsp70 transcripts during heat shock and recovery. (A) Third-instar larvae were incubated at various temperatures (25–42°C). Samples of total RNA (20 g each) isolated from the treated larvae, were fractionated by formaldehyde agarose gel electrophoresis, transferred to nylon membranes and hybridized with a $^{32}$P-labelled DNA fragment containing the coding region of the Cchsp70-B1 gene. (B) Third-instar larvae were heat shocked at 39°C for 30 min and then returned to 25°C for recovery. Samples of total RNA were extracted at various times after the end of heat shock and analysed as in (A). Numbers indicate recovery time in minutes.
et al., 1979) whereas the medfly genes are separated by long sequences (3.5–8 kb) and their restriction patterns seem to differ significantly. It has been postulated that gene conversion events maintain homogeneity among D. melanogaster hsp70 genes (Leigh-Brown & Ish-Horowicz, 1981). We speculate that the more dispersed organization of the hsp70 transcription units in C. capitata reduces the likelihood of gene conversion events, resulting in a more diverse restriction pattern among medfly hsp70 genes.

One of the medfly genes, Cchsp70-B1, was further characterized by sequencing. Cchsp70-B1 has a single open reading frame encoding a predicted protein of 70.07 kDa and shows high similarity, in the coding region, with the D. melanogaster and other eukaryotic hsp70 genes. Alignment of the deduced amino acid sequence to the amino acid sequences of D. melanogaster hsp70 proteins (Fig. 5) indicated a striking degree of conservation in the N-terminal region and a moderate conservation in the C-terminal region. Eukaryotic hsp70 proteins are highly conserved in the N-terminal region, corresponding to an ATPase domain, and less conserved in the C-terminal region (Lindquist, 1986; Feige & Polla, 1994, for reviews). The

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**Figure 7.** Chromosomal localisation of the Cchsp70-B1 gene. Salivary gland polytene chromosomes from untreated (25°C) and heat shock-treated (39°C for 30 min) 5-day-old larvae were hybridized with a DNA fragment containing the coding region of the Cchsp70-B1 gene. (A) and (C) show polytene chromosomes from untreated and heat-treated larvae respectively. (B) and (D) show in situ hybridization of polytene chromosomes from untreated and heat-treated larvae respectively. Arrows indicate the position of the 24C band or puff.
nucleotide sequence similarity observed in the coding region of medfly and *D. melanogaster* genes does not extend into the 5′ and 3′ flanking regions. However, similarly to *D. melanogaster* heat-inducible hsp70 genes, the Cchsp70-B1 has a rather long (approx. 200 bp) 5′ UTR which is very rich in adenosine residues (48%). This is a common feature of all *Drosophila* heat shock genes and seems to play an important role in the efficient translation of their mRNAs under heat shock conditions (Lindquist & Petersen, 1990, for a review). Furthermore, the 3′ UTR of the Cchsp70-B1 gene, like the *Drosophila* heat-inducible hsp70 genes, contains AT-rich sequences which seem to play a critical role in the degradation of the heat-shock messages under normal conditions and after recovery from a heat shock (Lindquist & Petersen, 1990, for a review). These AT-rich sequences are present in the 3′ UTR of transiently expressed genes and are best characterized by a single adenosine nucleotide followed by a polythymidine tract of three or more Ts (Shaw & Kamen, 1986). All the above characteristics of the Cchsp70-B1 gene, as well as the absence of intervening sequences, strongly support the conclusion that the cloned medfly hsp70 gene is a heat-shock cognate and not a heat shock cognate. The hsp70 cognate genes of *D. melanogaster* have intronss either in the coding regions or in the 5′ UTR (Craig *et al.*, 1983; Perkins *et al.*, 1990).

The Cchsp70-B1 gene has two characteristic HSEs proximal to the TATA box. A HSE was originally defined as a fourteen-base palindrome with a consensus sequence, CTnGAAnnTTChnAG (Pelham, 1982). Later studies showed that a functional HSE includes a minimum of three contiguous nGAAn units arranged in alternating orientation (Amin *et al.*, 1988). Both HSEs found in the Cchsp70-B1 gene match very well with Pelham’s consensus sequences (twelve matches of fourteen and thirteen matches of fourteen for the most proximal and less proximal HSE respectively) and they include three contiguous nGAAn units arranged in alternating orientation (Fig. 4). The variant unit, nGAAn, found in the most proximal HSE, is a very common variant of the HSEs in many heat shock genes and has been shown to be functional (Amin *et al.*, 1988). The positions of the Cchsp70-B1 HSEs, relative to the TATA box, are very similar to those of the heat-inducible hsp70 genes of *D. melanogaster*. Although *Drosophila* genes have two more HSEs, 154 and 224 bp upstream from the TATA box, it has been shown that only the first two proximal HSEs are necessary for optimal expression (Dudler & Travers, 1984; Simon & Lis, 1987). The sequence and the location of the Cchsp70-B1 HSEs suggest that they are functional. Whether these elements are sufficient or if additional sequences are required for optimal expression awaits detailed functional analysis of the 5′ flanking region of the gene.

*D. melanogaster* genes, especially those expressed in high levels, show considerable codon usage bias (Shield *et al.*, 1988). Among different *D. melanogaster* genes there is a correlation between degree of synonymous codon nonrandomness and levels of expression, suggesting that translational selection may be operating in *D. melanogaster* as in prokaryotes and yeast (Sharpe & Li, 1986; Sharp *et al.*, 1986). In contrast, several medfly and other tephritid highly expressed genes have a rather uniform synonymous codon usage (Rina & Savakis, 1991; He & Haymer, 1995). Our data are in agreement with the above reports. Unlike the *Drosophila* hsp70 genes which show a strong deficiency in A and T in the third position, the Cchsp70-B1 gene has a GC content in the third position similar to the total GC content.

The Northern analysis indicated that the maximal induction temperature for the medfly hsp70 genes is around 39°C, similar to that reported for the mosquito genes (Benedict *et al.*, 1993) and 4°C higher than the estimated optimum temperature in *D. melanogaster* (Vazquez *et al.*, 1993). This difference in the induction temperature between *Drosophila* and other insect species may be related to the observed low activity of the *Drosophila* hsp70 promoter in non-drosophilid insects. The evaluation of the activity of the Cchsp70 promoter is currently under investigation.

**Experimental procedures**

**Flies and materials**

A *C. capitata* strain maintained in our laboratory for 20 years was used for all experiments. The strain was originally established in the Benakeion Institute of Phytopathology (Athens, Greece) with flies from the Southern Peloponese (Greece) and Palermo (Italy). Insects were raised at 25°C, as previously described (Mintzas *et al.*, 1983). Under these conditions, embryonic development lasts about 50 h, the larval feeding stage lasts about 6 days, and the pupal stage lasts approximately 10 days. Embryos and larvae were synchronized by collecting freshly laid eggs within a 20 min period. A *D. melanogaster* wild-type strain, Canton-S, was also used in this study. 32P- and 35S-labelled nucleotides, DNA labelling kits (USB) and DNA sequencing kits (USB) were from Amersham. Restriction and modification enzymes were purchased from MinoTech (Heraklion, Greece), Boehringer Mannheim and Promega.

**General methods**

Genomic DNA from 24-h-old *C. capitata* embryos, *D. melanogaster* adults and *C. capitata* single flies was prepared according to the protocols described by Ashburner (1989). Preparation of phage DNA, agarose gel electrophoresis and blotting to Nytran-plus membranes (Schleicher and Schuell)
were carried out using standard procedures (Sambrook et al., 1989). DNA probes were prepared by random hexanucleotide priming (Feinberg & Vogelstein, 1983). Hybridizations of $^{32}$P-labelled probes to blotted DNA fragments were performed as described by Sambrook et al. (1989), at 65°C (D. melanogaster probes) or at 68–70°C (C. capitata probes) in 6× SSC, 0.5% SDS, 200 µg/ml sonicated heat-denatured salmon sperm DNA and 5 × Denhardt’s solution (Denhardt, 1966). Filters were washed twice at room temperature in 2 × SSC, 0.1% SDS for 15 min each, and twice at 55°C in 1% SSC, 0.1% SDS for 15 min each. Filters with homologous probes, were further washed at 65°C in 1% SSC, 0.1% SDS for 30 min. For Northern analysis, total RNA was isolated according to Schulze et al. (1989). RNA samples (20 µg) were separated by electrophoresis in 1.5% agarose gels containing formaldehyde and then transferred to Nytran-plus membranes (Sambrook et al., 1989). Hybridizations were performed at 42°C in 50% formamide, 5 × SSPE, 0.5% SDS, 200 µg/ml sonicated, heat-denatured salmon sperm DNA, and 2.5 × Denhardt’s solution. Filters were washed as described above for Southern analysis with homologous probes. Autoradiographic images were obtained either by exposing the filters to X-ray films or by using a phosphorimager SI (Molecular Dynamics). Preparation of plasmid DNA was carried out using the Wizard DNA purification system of Promega.

**Screening the genomic library**

A C. capitata EMBL4 genomic library (Rina & Savakis, 1991) was used. Approximately 300,000 plaques were screened, as described by Benton & Davis (1977), by hybridization at 65°C to a D. melanogaster hspt70 fragment of the genomic clone DMHS7P7D1, provided by S. Lindquist (University of Chicago). This clone corresponds to one of the D. melanogaster hspt70 distal genes, at the heat shock locus 87C1 (Ingolia et al., 1980). Hybridization and washing conditions were the same to those described for Southern analysis with heterologous probes.

**DNA sequencing and computer analysis**

Overlapping restriction fragments of medfly genomic DNA were subcloned into the pBluescript SK vector (Stratagene), and the DNA was sequenced from both strands according to Sanger et al. (1977) using the Sequenase version 2.0 DNA sequencing kit (USB). Each strand was sequenced at least twice and the data were analysed using the nucleic acid and protein sequence analysis program packages of PC/Gene (IntelliGenetics).

In situ hybridization

Squash preparations of salivary gland polytene chromosomes were made from untreated (25°C) and heat-shock treated (39°C for 30 min) 5-day-old larvae. Hybridization was performed as previously described (Zacharopoulou et al., 1992) by using biotin-16UTP (Boehringer Mannheim) for DNA labelling and the Avidin Elite kit (Vector Laboratories) for detection.

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