The EMBO Journal vol.4 no.11 pp.2971-2976, 1985

Identification of a sequence element in the promoter of the *Drosophila melanogaster hsp23* gene that is required for its heat activation

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Communicated by S.G.Clarkson

The expression of *Drosophila melanogaster hsp23*-*Escherichia coli* β-galactosidase hybrid genes containing different segments of the 5′ non-transcribed sequence of the *hsp23* gene has been examined at the RNA and protein levels in *Xenopus* oocytes. Transcription of the hybrid genes is initiated correctly. Mutant genes with *hsp23* gene promoter segments of at least 140 bp in length are strongly heat-activated while genes with shorter promoter segments are expressed constitutively and at low levels. This maps an element required for the heat-controlled expression of the *D. melanogaster hsp23* gene to a region, ~140 bp upstream from the start of the transcription site, which contains a sequence (CGAGAAGTTTCGTGT) that is closely related to the one responsible for the heat regulation of the *hsp70* gene. These findings demonstrate the importance of this regulatory sequence for a second *hsp* gene and support the notion that *hsp* genes are heat-regulated by a common mechanism. The functional element in the *hsp23* gene promoter is located >80 bp further upstream from the TATA box than the relevant element in the *hsp70* gene promoter. Even though other related sequences are present further upstream and downstream from the functional element, they play at most an auxiliary role in the regulation of *hsp23* gene expression.

Key words: gene expression/transcriptional and translational regulation/hybrid genes/*Xenopus* oocytes/deletion mutants

Introduction

The major *Drosophila melanogaster* heat shock protein (*hsp*) genes have been isolated and characterized in detail. They encode seven polypeptides of different mol. wts. and are expressed at high levels at temperatures of 35-37°C but are only weakly active at 25°C (for reviews, see Ashburner and Bonner, 1979; Schlesinger *et al.*, 1982).

Some of these *hsp* genes are not only stress-induced but are also active during specific stages of normal *Drosophila* development (Sirotkin and Davidson, 1982; Ireland *et al.*, 1982; Mason *et al.*, 1984; for review, see Voellmy, 1984). The *D. melanogaster hsp23* gene is active in late larval and early pupal stages. Expression of the gene has also been observed in freshly eclosed females. In addition, a number of drugs that are teratogenic in mammalian systems induce two of the *hsp* genes, the *hsp23* gene being one of them, in primary cultures of *D. melanogaster* embryonic cells (Buzin and Bournias-Vardiabasis, 1984). To learn more about the apparently complex regulation of the *hsp23* gene, we attempted the identification of DNA signals involved in the various aspects of its regulation. Here we describe the results of the first part of this study in which we have located and identified a sequence element which is required for the heat regulation of the gene.

Heat shock genes are found in a wide variety of eucaryotic (and even procaryotic) cell types. The structures of at least some of these genes as well as the mechanism of their regulation are highly conserved. The *D. melanogaster hsp70* gene is transcribed from the correct start site and in a heat-regulated fashion in mouse cells, *Xenopus* oocytes and monkey cells (Corces *et al.*, 1981; Voellmy and Rungger, 1982; Bienz and Pelham, 1982; Pelham, 1982; Mirault *et al.*, 1982). Analysis of promoter deletion mutants in these heterologous cells has led to the description of a short sequence element, located 48-62 bp upstream from the transcription start site of the *D. melanogaster hsp70* gene, which is responsible for its heat-regulated expression (Pelham, 1982; Bienz and Pelham, 1982; Mirault *et al.*, 1982). Elements resembling the *hsp70* gene sequence have been noticed in the promoter regions of many other *Drosophila hsp* genes and even of some recently isolated *hsp* genes from other eucaryotes (reviewed by Pelham, 1985), and comparison of these elements has led to the definition of a heat shock consensus sequence. These observations strongly suggest that sequence elements such as the one identified in the promoter of the *D. melanogaster hsp70* gene play a crucial role in the heat regulation of most or all *hsp* genes.

Even though they most likely have described the basic element for heat regulation, the above studies do not allow us to understand in detail the organization of some of the more unusual *hsp* gene promoters. The promoter region of the *D. melanogaster hsp27* gene appears to lack the typical heat shock motif altogether (Ingolia and Craig, 1981; Southgate *et al.*, 1983). While easily recognizable heat shock motifs are usually located immediately upstream from the TATA box, the corresponding region in the *hsp23* promoter contains a sequence which resembles the standard motif only weakly. At least five other sequence elements, many of which resemble the consensus sequence more closely than the latter element, are also present in the promoter region of the *hsp23* gene. To determine which (if any) of the various consensus sequence-like elements are responsible for the heat regulation of the *hsp23* gene, we have prepared *hsp23*-β-galactosidase hybrid genes with *hsp23* promoter segments of different lengths and have examined their expression at both the RNA and protein levels in *Xenopus* oocytes.

Results

Hybrid gene constructions

A *D. melanogaster* DNA fragment containing 1.5 kb of 5′ non-transcribed sequence of the *hsp23* gene, an intact *hsp23* gene RNA leader region and the first 45 *hsp23* codons was linked in phase to the truncated *Escherichia coli* β-galactosidase gene in plasmid MC1871. The resulting hybrid gene 23/26 is shown in Figure 1. To prepare *Bal*31 promoter deletion mutants, advantage was taken of the presence of a unique *Nco*I site ~600 bp

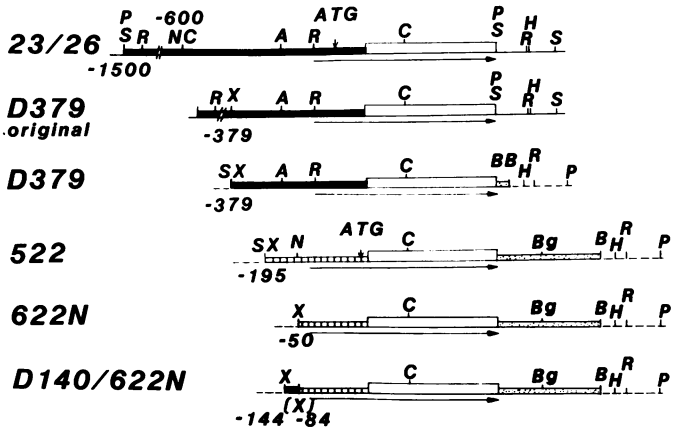

Fig. 1. Maps of *D. melanogaster hsp-E. coli* β-galactosidase hybrid genes. The numbers above and below the maps are distances in bp from the capping sites of the different genes except for −84 and −144 in the map of d140/622n which refer to the original coordinates of the *hsp23* gene fragment. An expanded scale has been used to represent the *hsp23* ■ and the *hsp70* ▥ gene promoter segments. The length of the *E. coli* β-galactosidase-coding region □ is 3 kbp. Horizontal arrows indicate the transcriptional orientation of the hybrid genes and provide estimates of the lengths of the transcripts. Other symbols: 3′ trailer sequences: ▨; pBR322 sequences: −; pSVOd sequences: ----; A: *Ava*I; B: *Bam*HI; BB: *Bam*HI/*Bgl*II; Bg: *Bgl*II; C: *Cla*I; H: *Hind*III; N: *Nru*I; NC: *Nco*I; P: *Pst*I; R: *Eco*RI; S: *Sal*I; SX: *Sal*I/*Xho*I; X: *Xho*I.

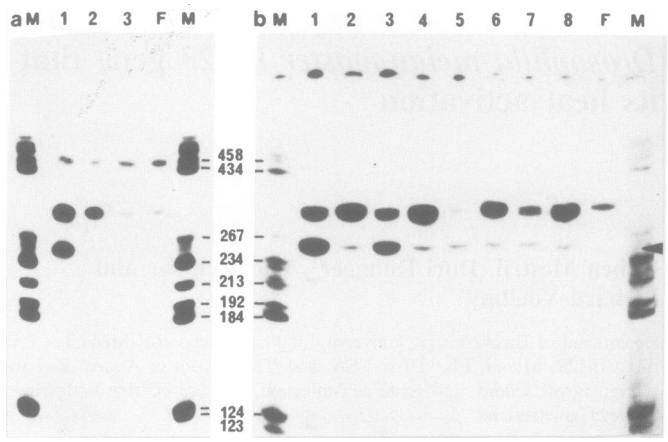

Fig. 2. S1 nuclease analysis of hybrid gene products made in *Xenopus* oocytes. A 320-bp probe fragment was isolated from *Xho*I/*Bam*HI-digested plasmid d63a (unrecloned) DNA. *Bam*HI cuts between the *hsp23* gene and the β-galactosidase segments. The fragment was end labeled and hybridized to 100 μg of total oocyte RNA. **A.** RNAs were from d379-containing, heat-treated **(lane 1)** or untreated **(lane 2)** oocytes or from uninjected, heat-treated oocytes **(lane 3). B.** RNAs were from heat-treated (odd-numbered lanes) or untreated (even numbers) oocytes that had been injected with d379 **(1,2)**, d186 **(3,4)**, d63a **(5,6)** or d139 DNAs **(7,8)**. F: probe fragment; M: end-labeled *Hae*III fragments of pBR322.

upstream from the previously established start of transcription site of the *hsp23* gene (Ingolia and Craig, 1981). An *Ava*I site located 147 bp upstream from the capping site was used to prepare mutant d147. Some of the mutants with very short promoter segments were made by *Bal*31 deletion from the unique *Sal*I site immediately upstream from the *hsp23* gene promoter segment in the d147 hybrid gene. To place them into a common vector background, the original mutant hybrid genes were recloned (see d379 in Figure 1, for example). The recloning step also served to add functional eucaryotic 3′-trailer sequences immediately downstream from all mutant genes derived from plasmid 23/26 (the β-galactosidase gene fragment in this plasmid is followed by pBR322 sequences).

The lengths of the promoter segments of all *hsp23* mutant hybrid genes are determined by direct DNA sequencing. The name of each mutant gene refers to the length in base pairs of its *hsp23* gene promoter region.

The *D. melanogaster hsp70*-β-galactosidase hybrid genes 522 and 622n have been described previously (Lawson *et al.*, 1984; Amin *et al.*, 1985), and their structures are shown in Figure 1. Mutant d140/622n is identical to the 622n gene except that a 61 bp long *hsp23* gene promoter segment had been inserted upstream of the truncated *hsp70* gene promoter.

The D. melanogaster hsp23 gene is transcribed correctly and in a heat-regulated fashion in Xenopus oocytes

Hsp23 hybrid genes such as d379 were microinjected into sets of 10−15 *Xenopus* oocytes. The oocytes were then either heat treated for 90 min at 36°C or kept at 21°C for the same length of time, and total RNA was prepared from them immediately afterwards. S1 nuclease protection experiments were carried out using as hybridization probe a 320-bp fragment isolated from the unrecloned mutant d63a as described in the caption to Figure 2. If transcription of the hybrid genes started at the correct site in *Xenopus* oocytes, a fragment of 257 bases should be protected from S1 nuclease digestion by RNA from oocytes which actively express injected *hsp23* hybrid genes.

RNA from heat-treated and untreated oocytes injected with d379 DNA and from uninjected, heat-treated oocytes was hybridized to the above probe. Following S1 nuclease digestion, the resistant material was analyzed on a 6% acrylamide/urea gel (Figure 2A). A strong band of the expected size of ~260 bases was observed when RNA from heat-treated oocytes containing the hybrid gene had been present in the hybridization reaction. Only small amounts of the same size fragment were protected by RNA from injected oocytes which had not been heat treated. RNA from uninjected oocytes did not hybridize to the probe. Variable amounts of full-length probe fragment were protected by RNAs from hybrid gene-injected, heat-treated or untreated oocytes. In most experiments RNA from untreated oocytes protected more full-length fragments than RNA from heat-treated oocytes. Such 'unspecific' readthrough transcription from upstream promoter sequences or from plasmid sequences has been observed before in oocyte expression experiments with other genes (Rungger *et al.*, 1981).

We conclude from these results that *Xenopus* oocytes recognize signal elements within the promoter region of the *D. melanogaster hsp23* gene. The correct transcription initiation site is being used effectively by the oocytes. Transcription from this site is strongly heat activated.

A sequence that is required for heat regulation is located ~140 bp upstream from the transcription start site of the hsp23 gene

The results discussed above indicate that the *Xenopus* oocyte transcription assay can be used to define elements involved in the heat regulation of the *D. melanogaster hsp23* gene. A number of different mutant genes was then analyzed by this assay. An example of the results obtained from these experiments is shown in Figure 2B, and a quantitative analysis of the heat inducibility of all mutant genes tested in several, independent experiments is presented in Table I.

Mutant genes d379, d186, d147 and d140 are strongly heat activated while mutants d139, d109, d84, d63a and b (two inde-

Table I. Heat activation of the transcription of different mutant hybrid genes

Mutant genes	Heat inducibility (fold)
d379	35 (7)
d186	27 (5)
d147	8.9 (2)
d140	7.7 (4)
d139	0.9 (5)
d109	1.0 (2)
d84	1.2 (4)
d63a	1.7 (4)
d63b	0.9 (4)

Autoradiographs of S1 mapping gels were scanned densitometrically. The number of independent experiments performed with each mutant gene is indicated in parentheses.

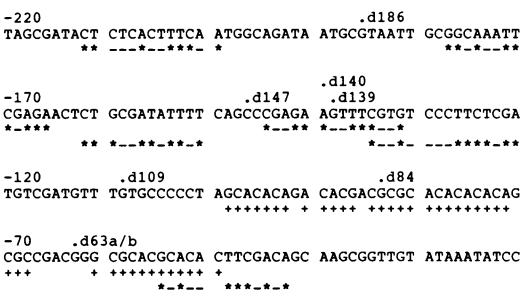

Fig. 3. The 5′ non-transcribed region of the *D. melanogaster hsp23* gene. The nucleotide sequence is from Southgate *et al.* (1983). The start of transcription site is at +1. The positions of the deletion end points in the different mutant genes are indicated above the nucleotide sequence. Regions of alternative purines and pyrimidines are marked by +, and matching nucleotides in regions containing sequences resembling the heat shock consensus element CTGGAATNTTCTAGA (Pelham, 1982) are marked by *.

pendently isolated mutant genes) are essentially not heat regulated. The latter mutant genes are transcribed at low levels, apparently from the correct transcription initiation site, in heat-treated as well as in untreated oocytes.

These results indicate that a regulatory sequence that is essential for the heat-controlled expression of the *hsp23* gene in oocytes is located ~140 bp upstream from the start of transcription site of the gene. This region (from −132 to −145) of the *hsp23* gene promoter contains a sequence element which matches the heat shock consensus sequence in eight of 14 positions (see Figure 3). We have noted that the d379 and d186 genes are two to three times more inducible than the d147 and d140 genes. We suggest that additional regulatory sequence elements located upstream from the one between positions −132 and −145 may play an auxiliary role in the regulation of the *hsp23* gene.

Oocytes containing hsp23 or hsp70 hybrid genes produce β-galactosidase in a heat-regulated fashion

Xenopus oocytes which had been injected with hybrid genes were heat treated at 36°C for 90 min or kept at 21°C for the same period. They were then routinely incubated at 21°C for another 15 h since it had been observed that essentially no β-galactosidase is produced during heat shock even though the hybrid genes are transcribed most actively during this period. The oocytes were subsequently lysed, and β-galactosidase activities were measured as described in Materials and methods. High levels of β-galactosidase were found in heat-treated oocytes containing the p522 *hsp70* hybrid gene but not in untreated or uninjected oocytes

Table II. Synthesis of β-galactosidase directed by *hsp70* and *hsp23* hybrid genes

Hybrid genes	Heat treatment	Relative β-galactosidase levels
522	+	1.00
522	−	0.03
622n	+	0.08
622n	−	0.05
d140/622n	+	0.79
d140/622n	−	0.04
d379	+	1.00 (6)
d379	−	0.15 (6)
d147	+	0.65 (5)
d147	−	0.14 (5)
d139	+	0.21 (4)
d139	−	0.09 (4)
d84	+	0.32 (2)
d84	−	0.18 (2)

Two independent sets of results which have been standardized independently are shown. Mean values are given for the expression data obtained with *hsp23* hybrid genes. The numbers of independent experiments performed with the different genes are in parentheses.

(Table II). The heat-regulated synthesis of β-galactosidase in *Xenopus* oocytes is clearly dependent on the presence of a functional *hsp* gene promoter in the *hsp70* hybrid genes; the 522 gene which contains 195 bp of *hsp70* gene promoter sequence (all essential promoter elements are included in this segment) produces ~30 times higher levels of β-galactosidase in heat-treated than in untreated oocytes (Table II). The *hsp70* hybrid gene in p622n which contains only 50 bp of promoter sequence and lacks the sequence that is required for heat regulation (Bienz and Pelham, 1982) directs the synthesis of ~15 times less β-galactosidase in heat-treated oocytes than the p522 gene. Slightly higher levels of β-galactosidase were measured in heat-treated than in untreated oocytes containing the 622n gene. We assume that the background activity produced by the 622n gene is due to read-through transcription or to some residual transcription from the correct start site (see Figure 2), and that the small heat-induced increase in this activity has to be explained by post-transcriptional events such as facilitated RNA transport during heat shock.

Seven-fold higher β-galactosidase levels were measured in heat-treated than in untreated oocytes containing the *hsp23* hybrid gene d379, and 4- to 5-fold higher levels with the d147 gene (Table II). Hybrid genes d139 and d84 are expressed at only 2-fold higher levels in heat-treated than in untreated oocytes. This weak apparent heat inducibility of the latter genes has most likely to be explained in the same fashion as that of the p622n *hsp70* hybrid gene. That the background activity would be more important in measurements of *hsp23* than of *hsp70* gene activity has to be expected considering the difference in the apparent strengths of the promoters of the two genes (considerably less β-galactosidase is produced by heat-treated oocytes containing the d379 gene than by oocytes that have been injected with the p522 gene). Thus, the results of these experiments which measured the expression of *hsp23* hybrid genes with promoter segments of different lengths at the protein level agree, at least qualitatively, with the data from the nuclease S1 assays described above.

The heat shock consensus-like sequence located immediately upstream from the TATA motif appears not to be an essential component of the hsp23 gene promoter

The heat shock regulatory sequence of the only *hsp* gene that has been studied in detail, the *D. melanogaster hsp70* gene, is

located immediately upstream from the TATA box (14 bp). A sequence which resembles the heat shock consensus sequence to at least some degree can be found in the corresponding position in the *hsp23* gene promoter (Figure 3). As has been shown before (see Table I and Figure 2), this sequence does not contain all the information required for the heat regulation of the *hsp23* gene. The above analysis of deletion mutants does not provide any information on whether the upstream regulatory sequence which is located 101 bp 5′ to the TATA motif is alone capable of rendering a gene heat-inducible or whether it can only function in conjunction with the consensus-like sequence element located further downstream.

To answer this question, mutant d140/622n was constructed (Figure 1). The promoter segment of the 622n *hsp70* hybrid gene does not include any heat shock consensus-like sequences, and the gene has been shown to produce only negligible levels of β-galactosidase in heat-treated or untreated oocytes (Table II). A 61 bp long promoter fragment (sequence from −84 to −144; see Figure 3) was isolated from mutant d140 which contains a short *hsp23* promoter region that does not extend beyond the upstream regulatory sequence element. This fragment was ligated, in the proper orientation, to the truncated promoter of the 622n gene. The upstream regulatory sequence of the *hsp23* gene promoter is the only heat shock consensus-like sequence in the resulting hybrid promoter and is located ~75 bp upstream from the TATA motif.

The d140/622n gene is strongly heat induced and is almost as active in heat-treated oocytes as the 522 gene which contains a functional, unmodified *hsp70* gene promoter (Table II). Thus, the upstream regulatory sequence of the *hsp23* gene is capable of rendering a gene heat-inducible even when located 75 bp upstream of the TATA box of the gene. It appears therefore that the consensus-like sequence next to the TATA box in the *hsp23* gene promoter is not required for gene regulation and may in fact not be functional.

Discussion

The experiments described here show that the *Xenopus* heat shock regulation system recognizes signal sequences in the promoter of the *D. melanogaster hsp23* gene. This finding contrasts with the observations of Pelham and Lewis (1983) and of Ayme *et al.* (1985) who found that the gene is hardly heat-inducible in COS monkey cells. The results of these authors raised the possibility that the expression of the gene might be controlled by *Drosophila*-specific signals, presumably in addition to one of the heat shock consensus-like elements (Pelham, 1985). It has even been suggested that the gene might be regulated by a mechanism that is altogether different from the one controlling the expression of most other *hsp* genes (Ayme *et al.*, 1985). That the transcription of the *D. melanogaster hsp23* gene is clearly heat-regulated in *Xenopus* argues strongly against the above interpretations of the COS cell expression experiments.

The 5′ non-transcribed region of the *hsp23* gene (Figure 3) contains a number of elements which resemble the heat shock consensus sequence (as defined in Pelham, 1982) to different degrees. Such sequences are found between positions −200 and −212, −166 and −178, −151 and −162, −132 and −145, −121 and −135 and −43 and −55. All of them match the consensus sequence in at least seven out of 14 positions. The element located at the position where the consensus sequence is normally found, i.e., 14−28 bp upstream from the TATA motif, fits the consensus sequence less well than most other elements present in the *hsp23* promoter region (seven matches only).

We report here that an *hsp23* hybrid gene with a 147 bp long promoter segment is strongly heat-induced while a similar gene with only 139 bp of promoter sequence is not heat-regulated (see Table I). This maps a sequence element required for the heat-regulated expression of the *hsp23* gene to a position corresponding exactly to that of one of the above consensus-like elements, the one between −132 and −145 (see Figure 3). That a mutant gene with only 140 bp of promoter sequence is still transcribed in a heat-controlled fashion is due to the fortuitous, almost perfect reconstruction of the functional regulatory sequence in this mutant by the synthetic *Xho*I linker that is present at the deletion end points of all mutant genes. The sequence at the 5′ end of the promoter region of mutant d140 is: cgaggAGTTTCGTGT (nucleotides matching the original promoter sequence are underlined). To our knowledge, our data represent the first direct demonstration that a consensus-like element is involved in the transcriptional regulation of a heat shock gene other than an *hsp70* gene. Thus, they provide the first direct *in vivo* evidence for the coordinate regulation of different *hsp* genes.

The promoter region of the *D. melanogaster hsp23* gene differs from that of the *hsp70* gene in that a basic promoter element is not located immediately upstream from the TATA box but 101 bp away from this motif. The organization of the promoter regions of most other *Drosophila hsp* genes resembles that of the *hsp70* genes; sequence elements that are closely related to the heat shock consensus sequence are found in positions corresponding to that of the *hsp70* gene element. Since in the *hsp23* gene promoter a stretch of sequence that fits the consensus sequence in at least seven out of 14 positions is located immediately 5′ to the TATA motif, we have investigated the possibility that the regulation of the gene may be based on a cooperative interaction between this element and the upstream element (at position −140). Our results do not provide any indication for the functional importance of the element near the TATA box; they therefore suggest that the upstream element alone is responsible for the heat-regulated transcription of the *hsp23* gene in *Xenopus* oocytes.

It has been noted that the region between the TATA motif and the upstream consensus-like sequence in the *hsp23* gene promoter contains a long stretch of alternating purines and pyrimidines which has the potential to form Z-DNA (located 49−99 bp upstream from the start of transcription site). Such sequence elements are absent from the promoter regions of most other *Drosophila hsp* genes. We have placed a segment of the *hsp23* gene promoter, that included the upstream consensus-like sequence but less than one third of the above region of simple sequence, in front of a truncated *hsp70* gene promoter. The upstream element which is located ~75 bp upstream from the TATA motif in the hybrid promoter is still capable of rendering the construct strongly heat-inducible. This result suggests that the long potential Z-DNA sequence, even though it may aid promoter function, is probably not an indispensable part of the *hsp23* gene promoter.

Studies of the herpes simplex virus thymidine kinase gene have revealed that the correct distance relationship between the TATA box and the next upstream promoter element is important for promoter function. Insertion of unrelated sequences of more than ~20 bp between the two transcription control sequences leads to a dramatic reduction in promoter activity (McKnight, 1982). Our data suggest that the distance between the heat shock regulatory sequence and the TATA motif is considerably less

crucial than it is for the signal sequences of the above gene: the heat shock regulatory sequence element appears to function at a distance of 14 as well as of 101 bp from the TATA box. This property, at least, it shares with typical enhancer sequences. It seems worth mentioning that the nucleotide sequences of a *Dictyostelium* heat shock promoter and of a *Xenopus hsp70* gene have been reported recently (Cappello *et al.*, 1984; Bienz, 1984). The closest consensus-like sequences in the promoter segments of the two genes are 140 and 120 bp, respectively, upstream from the TATA motif. The organization of these promoters, therefore, appears to resemble that of the *D. melanogaster hsp23* gene.

D. melanogaster hsp23 and *hsp70* hybrid genes direct the synthesis in heat-treated *Xenopus* oocytes of β-galactosidase in amounts that can be detected easily by the convenient colorimetric activity assay (*hsp70* hybrid gene activity can be measured in single oocytes). Our results indicate, in the case of the *hsp70* hybrid genes, that β-galactosidase synthesis is strictly dependent on the presence of functional *hsp* gene promoter segments in the hybrid genes. For the *hsp23* hybrid genes we have shown that a functional *hsp* gene promoter is required for high-level heat inducibility of β-galactosidase synthesis. This convenient assay for the transcriptional regulation of *hsp* gene expression and the fact that genes can be microinjected with relative ease make the *Xenopus* oocytes a powerful and convenient system for investigating the nature of the inducers of the heat shock response (membrane permeability problems are excluded) and for studying *in vivo* the mechanism of heat regulation.

Materials and methods

Construction of D. melanogaster hsp-E. coli β-galactosidase hybrid genes

Plasmid F4.17 contains, inserted into the unique *Pst*I site of vector pSVOd (Mellon *et al.*, 1981), a 3-kbp *D. melanogaster* DNA fragment which includes a complete *hsp23* gene and long stretches of flanking sequence [pF4.17 is identical to pF4 described in Southgate *et al.* (1983) except that the vector is pSVOd instead of pBR322]. F4.17 DNA was digested with *Pst*I, ends were made blunt by treatment with nuclease S1 and the material was digested further with *Pvu*II. A 1.75-kbp fragment including 1.5 kbp of 5′ non-transcribed sequence of the *D. melanogaster hsp23* gene, an intact RNA leader region and the first 45 *hsp23* codons was isolated and ligated to *Sma*I-cut pMC1871 DNA (Casadaban *et al.*, 1983) to give p23/26 (see Figure 1). To construct p23/22 (structure not shown in Figure 1), F4.17 DNA was digested with *Ava*I, ends were filled in by DNA polymerase large fragment and the material was digested with *Pvu*II. An isolated 390-bp fragment consisting of the 5′ end of the *hsp23* gene and of a segment of the promoter was ligated to *Sma*I-digested pMC1871 DNA. Colonies of the β-galactosidase-negative *E. coli* strain MC1061 containing either 23/26 or 23/22 produce detectable amounts of β-galactosidase on Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) indicator plates.

Plasmid 671S contains, in between the *Xma*III and the *Bam*HI sites of pSVOd, and in a direction opposite to that of the tetracycline resistance gene, the truncated β-galactosidase gene of pMC1871 followed by 2.3 kbp of 3′ non-translated and non-transcribed sequence of a *D. melanogaster hsp70* gene which includes the polyadenylation site. A *Sal*I linker had been introduced immediately 5′ of the β-galactosidase gene segment (see Lawson *et al.*, 1984). A mixture of p671S and p23/22 DNAs was digested with *Sal*I and *Cla*I, ligated and used for transformation. To identify d147, ampicillin-resistant, β-galactosidase-positive transformants were analyzed.

To prepare *Bal*31 deletion mutants, p23/26 DNA was digested with *Nco*I and d147 DNA with *Sal*I. Following digestion with *Bal*31 nuclease (Amin *et al.*, 1985), the DNAs were treated with DNA polymerase large fragment, *Xho*I linkers were added and, following digestion with *Xho*I, the material was religated. The resulting mutant genes were analyzed by restriction and, if considered useful, recloned: DNAs of mutants derived from p23/26 were digested with *Xho*I and *Sal*I and were ligated to *Sal*I-digested p132E3/6 DNA. Plasmid 132E3/6 contains, inserted in a counter-clockwise direction in the unique *Bam*HI site of pSVOd, a 3.3-kbp *Bgl*II segment which includes a complete *D. melanogaster hsp70* gene and flanking sequences (from plasmid 132E3; see Moran *et al.*, 1979). Digestion with *Sal*I separates all *hsp70* gene sequences from the vector segment except for the 250 bp long 3′ trailer sequence of the *hsp70* gene. The recloned *hsp23* hybrid genes were examined by extensive restriction digestion. An alternative recloning procedure was also used: DNAs of original *Bal*31 mutants were digested with *Xho*I and *Cla*I. Individual digests were ligated to *Sal*I/*Cla*I-cut p671S DNA, and the ligated material was used for transformation of *E. coli*. Ampicillin-resistant and β-galactosidase-producing colonies were selected for further characterization.

Plasmid 522 contains a functional *D. melanogaster hsp70*-β-galactosidase hybrid gene that includes a 195 bp long *hsp70* gene promoter segment (Lawson *et al.*, 1984). Plasmid 622n is essentially identical to p522 except that it contains a *hsp70* gene promoter fragment of only 50 bp (Amin *et al.*, 1985).

To construct the *hsp23/hsp70* hybrid gene d140/622n (see map in Figure 1), the original plasmid containing the d140 *hsp23* hybrid gene was digested with *Fnu*DII (cuts at position −83 in the *hsp23* gene promoter) and *Xho*I (cuts at the 5′ end of the promoter segment), and a 61 bp promoter segment (from −84 to −144) was isolated by electro-elution from a polyacrylamide gel. Plasmid 622n was digested with *Xho*I, sticky ends were filled in by DNA polymerase large fragment, and the DNA was then digested further with *Hind*III. A 6-kbp fragment which included the *hsp70* hybrid gene was obtained by electro-elution from an agarose gel. A 2.6-kbp fragment from a *Xho*I/*Hind*III double digest of p622n DNA was isolated likewise. The three isolated fragments were incubated with T4 DNA ligase, and the reaction mixture was used for transformation of *E. coli*. Recombinants were characterized by extensive restriction digestion.

DNA, RNA, DNA sequencing and S1 mapping

For the initial characterization of entire series of deletion mutants by restriction, DNAs were obtained by a rapid microscale procedure (Klein *et al.*, 1980). For all other experiments DNAs were prepared by CsCl banding from clear lysates by standard procedures. DNA sequence analysis was carried out by the method of Maxam and Gilbert (1977) except that fragments were isolated from low melt agarose gels (Maniatis *et al.*, 1982) and were labeled by DNA polymerase large fragment and [α-^{32}P]dXTP. Total RNA was prepared from batches of 10 *Xenopus* oocytes by a procedure involving proteinase K treatment of lysates and phenol extraction of the nucleic acids (Probst *et al.*, 1979). S1 mapping experiments were performed according to Weaver and Weissmann (1979) except that DNA fragments were not heated in pure formamide but resuspended and denatured directly in hybridization buffer.

Transient expression experiments

Groups of 10 − 15 *Xenopus* oocytes were injected (Rungger and Tuerler, 1978; Voellmy and Rungger, 1982) with 2 − 5 ng per oocyte of *hsp* hybrid gene-containing plasmids and 10 min later were either heat-treated at 36°C for 90 min or incubated at 21°C. RNA was then extracted as described above. For measurements of β-galactosidase activities, the oocytes were incubated further at 21°C for 15 h and were then lysed by pipetting in 700 μl (for 10 oocytes) of Z-buffer (Miller, 1972). The lysates were spun for 15 min in an Eppendorf microfuge, and 400 μl aliquots of the supernatants were added to 100 μl of 4 mg/ml *o*-nitrophenyl-β-D-galactopyranoside in Z-buffer. Reactions were for 2 − 20 h at 35°C. They were stopped by 250 μl of 1 N Na_2CO_3. After centrifugation to remove insoluble material, the absorbance of the mixtures at 420 and 550 nm was determined and β-galactosidase activities were calculated by the formula OD420 − 1.1 × OD550.

Acknowledgements

We thank L.Muster, K.Nemeth, H.Klapper and J.Amin for their help with some of the experiments and artwork and some of our colleagues for animals and for the use of their equipment. This work was supported by grants from the Swiss National Foundation (3.244-082) to D.R., and from NIH (GM 31125) to R.V.

References

Amin,J., Mestril,R., Lawson,R., Klapper,H. and Voellmy,R. (1985) *Mol. Cell. Biol.*, **5**, 197-203.
Ashburner,M. and Bonner,J.J. (1979) *Cell*, **17**, 241-254.
Ayme,A., Southgate,R. and Tissieres,A. (1985) *J. Mol. Biol.*, **182**, 469-475.
Bienz,M. (1984) *EMBO J.*, **3**, 2477-2483.
Bienz,M. and Pelham,H.R.B. (1982) *EMBO J.*, **1**, 1583-1588.
Buzin,C.H. and Bournias-Vardiabasis,N. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 4075-4079.
Cappello,J., Zuker,C. and Lodish,H.F. (1984) *Mol. Cell. Biol.*, **4**, 591-598.
Casadaban,M.J., Martinez-Arias,A., Shapira,S.K. and Chou,J. (1983) *Methods Enzymol.*, **100**, 293-308.
Corces,V., Pellicer,A., Axel,R. and Meselson,M. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 7038-7042.
Ingolia,T.D. and Craig,E.A. (1981) *Nucleic Acids Res.*, **9**, 1627-1642.
Ireland,R., Berger,E., Sirotkin,K., Yund,M., Osterbur,D. and Fristrom,J. (1982) *Dev. Biol.*, **93**, 498-507.
Klein,R., Selsing,E. and Wells,R. (1980) *Plasmid*, **3**, 88-91.
Lawson,R., Mestril,R., Schiller,P. and Voellmy,R. (1984) *Mol. Gen. Genet.*, **198**, 116-124.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
Mason,P., Hall,L. and Gausz,J. (1984) *Mol. Gen. Genet.*, **194**, 73-78.
Maxam,A. and Gilbert,W. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 560-564.

McKnight,S.L. (1982) *Cell*, **31**, 355-365.
Mellon,P., Parker,V., Gluzman,Y. and Maniatis,T. (1981) *Cell*, **27**, 279-288.
Miller,J. (1972) *Experiments in Molecular Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Mirault,M.-E., Southgate,R. and Delwart,E. (1982) *EMBO J.*, **1**, 1279-1285.
Moran,L., Mirault,M.-E., Tissieres,A., Lis,J., Schedl,P., Artavanis-Tsakonas,S. and Gehring,W. (1979) *Cell*, **17**, 1-8.
Pelham,H.R.B. (1982) *Cell*, **30**, 517-528.
Pelham,H. (1985) *Trends Genet.*, **1**, 31-34.
Pelham,H.R.B. and Lewis,M.J. (1983) in Hamer,D.H. and Rosenberg,M.J. (eds.), *Gene Expression*, Alan R.Liss, Inc., NY, pp. 75-85.
Probst,E., Kressmann,A. and Birnstiel,M.L. (1979) *J. Mol. Biol.*, **135**, 709-732.
Rungger,D. and Tuerler,H. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 6073-6077.
Rungger,D., Matthias,P.D. and Huber,J.P. (1981) in Schweiger,H.G. (ed.), *International Cell Biology 1980–1981*, Springer, Berlin, pp. 28-32.
Schlesinger,M., Ashburner,M. and Tissieres,A. (1982) *Heat Shock from Bacteria to Man*, published by Cold Spring Harbor Laboratory Press, NY.
Sirotkin,K. and Davidson,N. (1982) *Dev. Biol.*, **89**, 196-210.
Southgate,R., Ayme,A. and Voellmy,R. (1983) *J. Mol. Biol.*, **165**, 35-57.
Voellmy,R. and Rungger,D. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1776-1780.
Voellmy,R. (1984) *BioEssays*, **1**, 213-217.
Weaver,R.F. and Weissmann,C. (1979) *Nucleic Acids Res.*, **6**, 1175-1192.

Received on 29 April 1985; revised on 1 August 1985