Hsp28^{stl}: A *P*-Element Insertion Mutation That Alters the Expression of a Heat Shock Gene in *Drosophila melanogaster*

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ABSTRACT

We have identified and cloned a mutant allele of the small heat shock gene *Hsp28* of *Drosophila melanogaster*. This allele, which we have called *Hsp28*^{stl}, produces small amounts of a single aberrantly large, heat-inducible transcript in heat-shocked flies, while a normal-sized *Hsp28* transcript is present only in fertile females. No *Hsp28* transcript at all is detected in mutant prepupae, a stage when wild-type flies show high levels of *Hsp28* RNA. We have cloned the *Hsp28*^{stl} allele, and have found that a 1.3-kb defective *P*-element is present 5′ to *Hsp28* in the mutant line. The site of *P*-element insertion lies between the *Hsp28* "TATA box" sequence and the *Hsp28* RNA cap site; in contrast to previously described *P*-element insertions, the element at *Hsp28*^{stl} is flanked by a two base pair duplication of the insertional target sequence. The results suggest that this insert may separate elements regulating heat-inducible and developmental expression of *Hsp28*, leading to the different patterns of transcription observed.

TRANSPOSON mutagenesis has been shown to be the source of many spontaneously arising regulatory mutations in a variety of organisms including yeast (ERREDE *et al.* 1980; WILLIAMSON, YOUNG and CIRIACY 1981), plants (BURR and BURR 1983; DORING *et al.* 1984), vertebrates (HAYWARD, NEEL and ASTRIN 1981; JENKINS *et al.* 1981; NEEL *et al.* 1982) and in particular Drosophila (SNYDER *et al.* 1982; BENDER *et al.* 1983; MCGINNIS, SHERMOEN and BECKENDORF 1983; SCOTT *et al.* 1983; TSUBOTA, ASHBURNER and SCHEDL 1985; ZACHAR *et al.* 1985; COTÉ *et al.* 1986). In Drosophila, the recognition that extensive transposable element mobilization is the basis for the high levels of mutation and chromosome rearrangement that accompany hybrid dysgenesis has provided a mechanism for the efficient generation of mutant alleles, useful in elucidating the basis for transcriptional regulation. It has been suggested that transposon mutagenesis may be more efficient for the generation of regulatory mutations than EMS (TSUBOTA, ASHBURNER and SCHEDL 1985), perhaps because *cis*-acting regulatory elements are relatively insensitive to single base changes. It has recently been shown that additional mutational variation induced by *P*-element mobilization in a dysgenic population results in an accelerated response to selection much greater than that seen in similar experiments using X-ray irradiation (MACKAY, 1985). The more drastic mutagenic effect of transposable elements may be due to the fact that many transposable elements contain transcription units; thus, insertion may interfere with or alter the transcription of nearby genes by the direct effect of transcription within the element itself (ZACHAR *et al.* 1985), or by the introduction of novel chromatin structure associated with the element (EISSENBERG *et al.* 1984; EISSENBERG and ELGIN 1986).

In the course of *P*-element mediated transformation experiments aimed at elucidating the relationship between chromatin structure and gene expression at the small heat shock gene cluster at 67B in *Drosophila melanogaster*, we induced a mutation of *Hsp28*, one of the heat-inducible genes within this cluster. We have cloned the mutant *Hsp28* allele, which we have called *Hsp28*^{stl}. Sequence analysis of the clones *Hsp28*^{stl} allele revealed a defective *P*-element insertion between the *Hsp28* "TATA box" element and the mRNA cap site sequence. The *P*-element insert is flanked by a dinucleotide duplication of the target site. The introduction of *P*-element sequences 5′ to *Hsp28* dramatically perturbs transcription at the locus, differentially affecting heat shock and developmental regulation in *Hsp28*^{stl} flies.

MATERIALS AND METHODS

Fly stocks and maintenance: The *Hsp28*^{stl}-bearing chromosome was originally identified in a stock carrying an *X*-linked transduced copy of *Hsp28* (EISSENBERG and ELGIN 1986). This stock had been transformed by co-injection of a plasmid consisting of the *Hsp28* gene cloned into the *P*-element transforming vector Carnegie 4 (RUBIN and SPRADLING 1983) and the intact *P*-element-bearing plasmid pπ25.1 (O'HARE and RUBIN 1983). The distal part of 3L bearing *Hsp28*^{stl} and a linked, spontaneously arising mutant allele of *sepia* were crossed onto the *ru cu ca* multiply marked third chromosome (LINDSLEY and GRELL 1968). *Hsp28*^{stl} was then maintained as a homozygous stock marked with *se th st cu sr e* and *ca*. These flies show somewhat reduced fertility when maintained at 25°, but survival and fertility

are not discernibly affected by a 45-min heat shock at 37°. Flies were maintained at room temperature on a cornmeal-agar medium in half-pint disposable bottles. Flies were heat shocked by placing them in 1.5-ml Eppendorf tubes at 37° for 45 min.

Cloning of the *Hsp28^{stl}* allele: DNA from the *Hsp28^{stl}* line was prepared by flash-freezing adult flies in liquid nitrogen, and then grinding the flies to a powder using a prechilled pestle and a mortar filled with liquid nitrogen. After the nitrogen evaporated, the fly powder was dissolved in homogenization buffer (0.2 M sucrose, 0.1 M Tris-HCl, pH 9.2, 50 mM EDTA, 0.5% SDS), the DNA extracted twice with phenol and twice with chloroform, and then ethanol precipitated. Since genomic restriction analysis had shown that *Hsp28^{stl}* is contained on a *ca.* 12.5-kb *Bam*HI restriction fragment, approximately 20 μg of *Hsp28^{stl}* DNA were digested to completion with *Bam*HI and size-fractionated on a 5-ml Sephacryl S-1000 (Sigma) column equilibrated in 10 mM Tris-HCl (pH 7.0)-1 mM EDTA before use. Column fractions were analyzed by agarose gel electrophoresis, and fractions containing DNA of between 10 and 15 kb in size were pooled. Approximately 2 μg of size-selected genomic DNA were ligated overnight to approximately 1 μg of EMBL 3λ phage arms (predigested with *Bam*HI, Vector Cloning Labs, San Diego) according to manufacturer's directions. A sample of the completed ligation reaction was then packaged using commercial packaging extract (GigaPak, Vector Cloning Labs), and used to infect restrictive host bacteria according to the manufacturer's directions. Infected bacteria were plated in soft agar, and the phages were screened by plaque hybridization (Benton and Davis 1977) using the insert fragment of plasmid 88B13 which contains *Hsp28* (Corces *et al.* 1980) as a hybridization probe. Plaques which hybridized strongly were selected, and phage DNA was prepared from a 1-L cleared lysate by polyethylene glycol (PEG) precipitation of intact phage (7% PEG weight/volume), followed by phenol extraction and ethanol precipitation of phage DNA. Restriction fragment subclones from phage DNA were prepared by electroelution of the desired fragment from an agarose gel, followed by ligation into the appropriate restriction sites of pUC 13 (Messing 1983).

DNA sequencing: Subcloned fragments bearing regions of interest were sequenced directly from plasmid DNA. Two micrograms of DNA were denatured in 0.2 N NaOH, 0.2 mM EDTA for 5 min at room temperature, then made 0.3 N with sodium acetate (pH 5.0), and the DNA was precipitated with two volumes of ethanol. The plasmid template was annealed to either direct or reverse sequencing primer (New England Biolabs) and sequenced by the dideoxy chain termination method of Sanger, Nicklen and Coulson (1977).

Northern blot analysis: Total nucleic acids were purified from individual flies of the stage indicated, essentially according to the method of Meyerowitz and Hogness (1982), as modified by Steiner, Eissenberg and Elgin (1984). Samples were electrophoresed in agarose-formaldehyde gels according to Lehrach *et al.* (1977). After electrophoresis, the gels were soaked in 20 × SSC for 30 min with one change, and blotted to nitrocellulose paper in 20 × SSC.

DNase I digestion of larval nuclei: Larval nuclear preparations and DNase I digestions were performed essentially as described in Eissenberg and Lucchesi (1983). Purified DNA was then digested to completion with restriction enzyme and electrophoresed in agarose-TAE gels (Hayward and Smith 1972). DNA was then denatured *in situ* by soaking the gel in 0.5 N NaOH-1.5 N NaCl for 45 min, neutralized in 0.5 M Tris-HCl (pH 7.0)-3 N NaCl for 30 min, and blotted to nitrocellulose paper in 20 × SSC.

Hybridization analysis of Northern blots, Southern blots and plaque filter lifts: Probes used in the hybridization analysis of nucleic acids immobilized on nitrocellulose paper were labeled to high specific activity ($1–3 \times 10^8$ cpm/μg) by the incorporation of ^{32}P-labeled nucleotides using the nick-translation method of Maniatis, Jeffrey and Kleid (1975). Labeled probe DNA was separated from unincorporated nucleotides by Sephadex G-100 chromatography. Filters were baked, prehybridized and hybridized to labeled probes essentially as described in Wahl, Stern and Stark (1979), except that prehybridizations and hybridizations were at 65° without formamide. Hybridized filters were subsequently washed as described, dried and exposed to XAR 5 X-ray film (Kodak) at −80°, using a Chronex Lightning Plus intensifying screen (DuPont).

RESULTS

During the course of our characterization of *P*-element transduced *Hsp28* genes, we identified a line of transformed flies which had spontaneously mutated to a *sepia* phenotype. Southern and Northern blot analysis showed an aberrant genomic restriction digestion pattern at the 67B locus, and an absence of a normal heat-inducible *Hsp28* transcript in some of these flies. As the absence of *Hsp28* RNA was not strictly correlated with the eye color phenotype, we assume that the two traits are merely chromosomally linked [*sepia* appears to lie between 66A and 66D; Lindsley and Grell (1968)] and not causally related.

Comparison of the genomic restriction enzyme digestion patterns at 67B in wild-type and the *sepia-Hsp28$^-$* (hereafter referred to by the name of the mutant allele *Hsp28^{stl}*) flies suggested that an insertion of 1.3 kb of DNA had occurred in the vicinity of *Hsp28*. In particular, the *Eco*RI fragment containing *Hsp28* (Figure 1A) is 1.3 kb larger in *Hsp28^{stl}* flies (data not shown). In order to map more precisely the site of this insertion, we exploited the strong sequence-preferential cleavage of DNA by micrococcal nuclease (Keene and Elgin 1981, 1984; Flick, Eissenberg and Elgin 1986). Sites of preferential micrococcal nuclease cleavage occur at an average of every 200 bp in eukaryotic noncoding DNA, so that an insertion of 1.3 kb of heterologous DNA should introduce an easily discernible disruption of the wild-type pattern. Purified genomic DNA from both wild-type and *Hsp28^{stl}* flies was digested briefly with micrococcal nuclease, digested to completion with *Bam*HI, and electrophoresed in 0.8% agarose gel. The DNA was then analyzed by the technique of indirect end-labeling (Wu 1980; Nedospasev and Georgiev 1980). Figure 1B shows the results of this analysis. The pattern of micrococcal nuclease cleavage is perfectly aligned in the wild-type and *Hsp28^{stl}* samples up to a position corresponding to the 5′ end of *Hsp28*. An obvious gap appears in the *Hsp28^{stl}* pattern at this point, followed by a resumption of the wild-type frag-

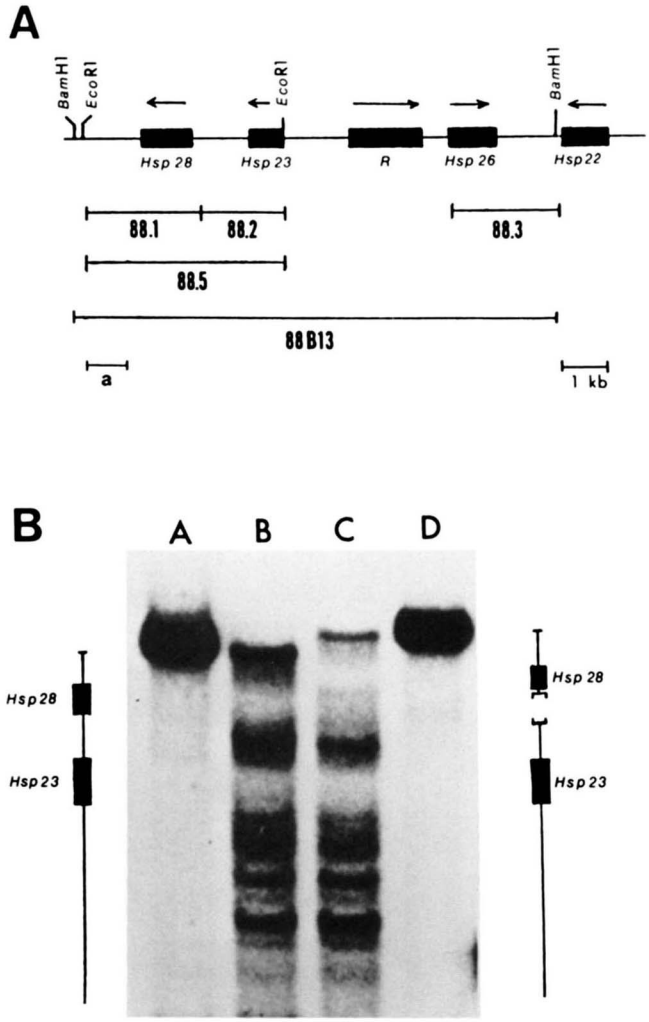

FIGURE 1.—A, Map of the 67B heat shock gene cluster. Genes are indicated by blocks, with arrows above showing the direction of transcription. Lines below the map indicate the relative positions of sequences used as probes in this paper. B, Positioning of the insertion element at 67B in the *Hsp28*^{stl} line using partial micrococcal nuclease digestion mapping. Genomic DNA was subjected to partial micrococcal nuclease digestion and subsequent indirect end labeling analysis as described in the text. The hybridization probe was 88.3 (CORCES *et al.* 1980). Lane A: Oregon R DNA digested with *Bam*HI. Lane B: Oregon R DNA digested briefly with micrococcal nuclease, then digested to completion with *Bam*HI. Lane C: *Hsp28*^{stl} genomic DNA digested briefly with micrococcal nuclease, then digested to completion with *Bam*HI. Lane D: *Hsp28*^{stl} genomic DNA, digested with *Bam*HI. Maps flanking the figure indicate the relative positions of genes within the region under analysis. A gap in the right-hand map marks the position of the discontinuity in the micrococcal nuclease digestion pattern, which indicates the position of the inserted sequence.

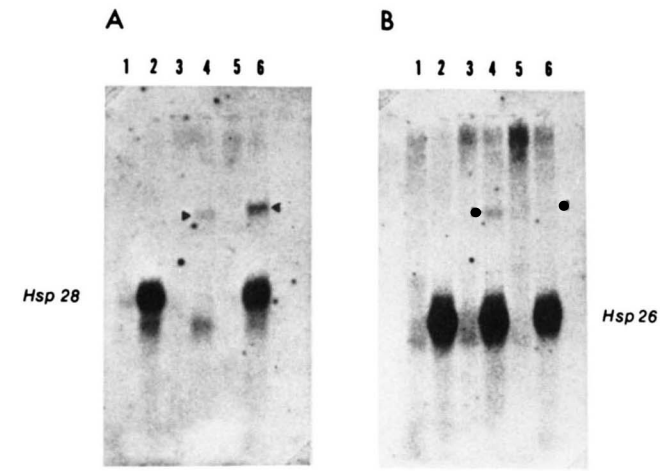

FIGURE 2.—The novel heat shock RNA of *Hsp28*^{stl} is homologous to both *Hsp28* and pπ25.1. Panel A, Northern blot analysis of heat shock (lanes 2, 4 and 6) and non-heat-shock (lanes 1, 3 and 5) RNA from Oregon R (lanes 1 and 2) adult males, *Hsp28*^{stl} (lanes 3 and 4) adult males, and adult males of a transformed line (lanes 5 and 6). An equivalent amount of each RNA sample was electrophoresed in a 1.5% agarose-formaldehyde gel. The hybridization probe is 88.1 (CORCES *et al.* 1980), a plasmid with homology to *Hsp28*. The transformed line HSAd010 contains a transduced copy of *Hsp28* tagged with 1.4 kb of heterologous sequence inserted into the protein-coding region of the gene (EISSENBERG and ELGIN 1986). The position of *Hsp28* RNA is indicated to the left of the figure. Arrowheads indicate the relative position of the heat-inducible *Hsp28*^{stl} and transduced *Hsp28* RNA (lanes 4 and 6, respectively). Hybridization in the position of *Hsp26* (lowest band) represents residual signal from previous hybridization of the filter with probe 88.3. Panel B, Same as panel A, except that the Northern blot was hybridized using the *P*-element bearing plasmid pπ25.1 and plasmid 88.3 (CORCES *et al.* 1980). Probe 88.3 carries sequences homologous to *Hsp26*, and was used to indicate both the position of the heat shock RNA and relative levels of that heat-induced RNA. The filled arrowhead indicates the *P*-homologous transcript in *Hsp28*^{stl}, while the open arrowhead indicates the position of the transduced *Hsp28* RNA, which has no homology to *P*-element. Hybridization signal near the top of the blot represents hybridization to residual high molecular weight DNA.

ment pattern associated with the *Hsp28* coding region and 3′ sequences. Thus, the 1.3-kb insert is located immediately 5′ to *Hsp28*. The nature of this insertion is suggested by the following observations. The insertion-bearing chromosome was identified in a line containing active *P*-elements (introduced by injection of pπ25.1) which yielded several spontaneous visible mutations as well as *sepia*. *P*-element DNA is present at 67B, as demonstrated by *in situ* hybridization (data not shown). Since the full-sized *P*-element is 2.9 kb, we suspected that the 1.3-kb insertion was probably a defective *P*-element.

Figure 2A shows a comparison, by Northern blot analysis, of heat shock and nonheat shock RNA prepared from wild-type and mutant flies. Even on long autoradiographic exposure, no normal *Hsp28* RNA is detectable in mutant flies after heat shock. A novel RNA appears in these flies, however, which is heat induced (compare lanes 3 and 4) and not present in wild-type heat-shocked flies (compare lanes 2 and 4). This RNA is slightly smaller than an *Hsp28*-adenovirus fusion transcript which contains a 1.4-kb adenovirus insert (compare lanes 4 and 6).

An RNA of this size would be expected if the 1.3-kb insert 5′ to *Hsp28* were cotranscribed with the *Hsp28* gene as a fusion transcript. To test this idea, we hybridized the blot shown in panel A with a *P*-

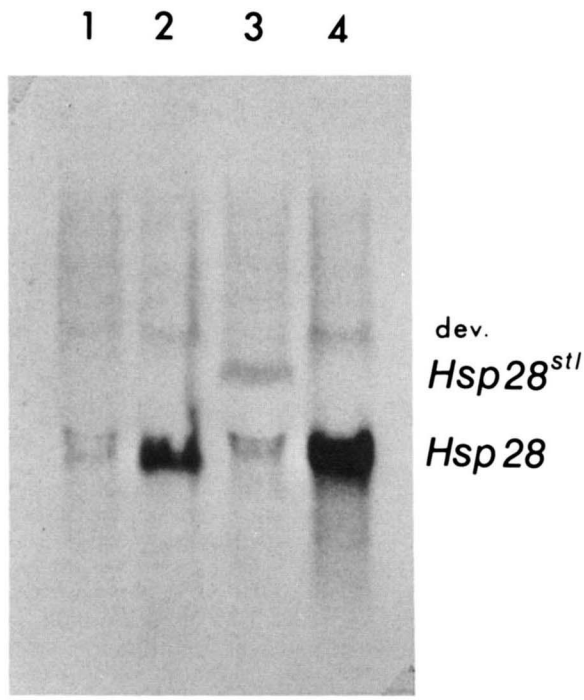

FIGURE 3.—Developmental expression of *Hsp28*^{stl}: fertile females. Non-heat-shock RNA was prepared from fertile *Hsp28*^{stl} females (lane 1) and fertile Oregon R females (lane 2). Equivalent amounts of RNA were electrophoresed in a 1% agarose formaldehyde gel and analyzed using Northern blots. Lanes 3 and 4 show heat shock RNA from *Hsp28*^{stl} female and Oregon R females, respectively. The relative positions of *Hsp28* and the *Hsp28*^{stl} heat-shock specific transcripts are indicated to the left of the figure. "dev." indicates the position of a developmentally regulated transcript encoded downstream of *Hsp28*, detectable with this hybridization probe (J. C. EISSENBERG and S. C. R. ELGIN, unpublished data). The hybridization probe was 88.1 (CORCES *et al.* 1980).

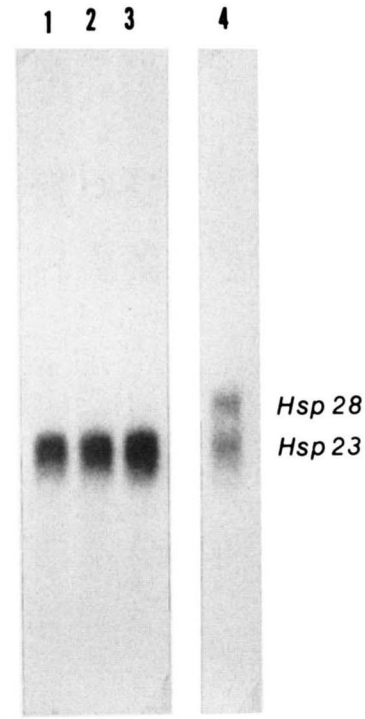

FIGURE 4.—Developmental expression on *Hsp28*^{stl}: white prepupae. Non-heat-shock RNA was prepared from a later third instar larva (lane 1) and white prepupae (lanes 2 and 3) of the *Hsp28*^{stl} line. Lane 4: non-heat-shock RNA from an Oregon R prepupa. Equal amounts of RNA were electrophoresed on the same 1% agarose-formaldehyde gel. The hybridization probe was 88.5 (CORCES *et al.* 1980). Lanes 1–3 were overexposed relative to lane 4 in order to detect possible low abundance *Hsp28* transcripts.

element-specific probe (Figure 2B). The larger heat-inducible RNA is homologous to both the *Hsp28* and *P*-element probes. Note that another line containing many *P*-element copies, but not the *Hsp28*^{stl} allele, contains no such heat-inducible *P*-element homologous transcript (compare lanes 4 and 6 in Figure 2B). The quantity of the larger *Hsp28* RNA induced by heat shock in the mutant is dramatically lower than the amount of normal *Hsp28* RNA induced by heat shock in wild-type flies, perhaps as a consequence of reduced transcription, reduced stability of the larger transcript, or both.

The small heat shock genes at 67B are subject to developmental regulation. *Hsp28* is normally expressed at high levels in white prepupae and in the ovaries of fertile adult females (CHENEY and SHEARN 1983; ZIMMERMAN, PETRI and MESELSON 1983). A comparison of RNA prepared from fertile females of wild-type and *Hsp28*^{stl} lines, using Northern blots, is shown in Figure 3. In contrast to heat-shocked adult male flies, low levels of *Hsp28* RNA of the normal size are detectable from non-heat-shock *Hsp28*^{stl} fertile females. No larger RNA of the size detectable after heat shock in this line is seen in the non-heat-shocked females. The normal sized *Hsp28* RNA present in samples from heat-shocked *Hsp28*^{stl} flies probably reflects stable non-heat-shock RNA since (1) no induction is apparent upon heat treatment, and (b) this transcript is not visible in RNA from heat-shocked *Hsp28*^{stl} males (Figure 2) or newly enclosed *Hsp28*^{stl} females (data not shown). A similar Northern blot analysis of RNA prepared from wild-type and *Hsp28*^{stl} white prepupae is shown in Figure 4. In this case, no *Hsp28* RNA of any size from *Hsp28*^{stl} flies is detectable, even after long exposures.

The different effects of the insert DNA on the expression of *Hsp28*^{stl} under different conditions and at different developmental times suggested to us that the insertion lay in an important regulatory region. We therefore cloned the *Hsp28*^{stl} allele into the phage lambda vector EMBL 3 and sequenced the region of the insertion site from plasmid subclones. A schematic map of the *Hsp28*^{stl} locus, showing the results of this analysis, is presented in Figure 5. The following results were obtained from the sequence analysis. First, the insertion occurs between the normal TATA box and cap site for *Hsp28*, effectively separating these two signals, originally 30 bp apart, by 1.3 kb. Only 2 bp of the insertion target are duplicated on each side of the insert. Second, sequences for several dozen base

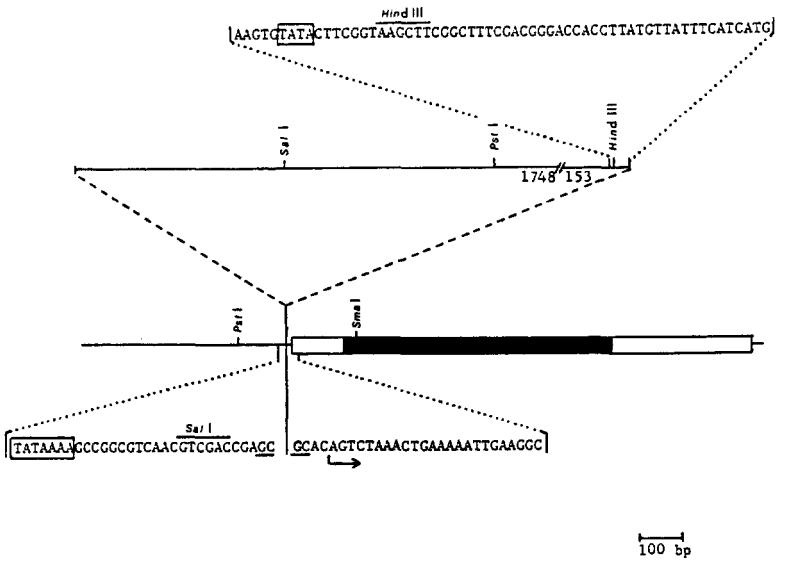

FIGURE 5.—A schematic map showing the molecular organization of the *Hsp28*^{stl} allele. The extent of the wild-type *Hsp28* transcript is indicated by the blocked region, with the filled area indicating the protein-coding sequences. A long vertical line immediately to the left of the gene indicates the position of the defective *P*-element. The *P*-element is represented by a line above the map, with a break in the line indicating the position of the internal deletion in the defective *P*-element. The last 60 nucleotides at the proximal end of the insert are shown above the *P*-element map with the *P*-element TATA motif indicated by a box. Below the map is shown the sequence flanking the insert. The wild-type TATA motif is indicated by a box, while the wild-type *Hsp28* cap site and direction of transcription are indicated by an arrow. Nucleotides duplicated at the *P*-element insertion site are underlined.

pairs at each end of the insert correspond exactly to those found at the termini of the published *P*-element sequence (O'HARE and RUBIN 1983). The *P*-element copy here is oriented in the opposite direction (with respect to its normal transcriptional polarity) to the adjacent *Hsp28* gene. A deletion of 1595 bp of *P*-element sequence beginning at position 153 was revealed by sequence analysis (data not shown) and comparison to the published sequence (O'HARE and RUBIN 1983). This deletion would completely account for the size difference between this element and the full-sized 2.9-kb element. Finally, the orientation of the *P*-element places a "TATA" motif 60 bp upstream to the normal *Hsp28* cap site. This sequence, which also reads "TATA" 5′ to 3′ on the other strand, is the presumptive promoter for the wild-type *P*-element transcript. Note, then, that the direction of normal *P*-element transcription is divergent from *Hsp28* transcription.

A detailed analysis of the chromatin structure of the wild-type *Hsp28* locus has recently been presented revealing a complex pattern of DNA-protein interactions 5′ to this gene (CARTWRIGHT and ELGIN 1986). In addition to the introduction of 1.3 kb of novel DNA sequence 5′ to *Hsp28* in *Hsp28*^{stl} flies, the effect of the *P*-element insertions could be to alter the chromatin structure upstream of *Hsp28* in such a way so as to interfere with DNA-protein interactions required for properly regulated expression. Since *Hsp28*^{stl} shows a different expression pattern under each regulatory program, we were interested in determining whether features of chromatin structure 5′ to *Hsp28* were also dramatically altered by the transposable element insertion. Nuclei prepared from wild-type and *Hsp28*^{stl} third instar larvae were digested with DNase I to various extents, after which the DNA was purified and subjected to indirect end labeling analysis. Figure 6A shows the results of such an analysis. The pattern of DNase I hypersensitivity in wild-type larvae agrees well with the general pattern previously reported for non-heat-shock tissue culture cells (CARTWRIGHT and ELGIN 1986). In the chromatin of the *Hsp28*^{stl} larvae, the pattern of DNase I hypersensitive sites within the wild-type sequences upstream of the *P*-element insert does not appear at this level of resolution to be altered. An additional prominent DNase hypersensitive site is associated with the distal terminal sequences of the *P*-element insert as shown in Figure 6B.

DISCUSSION

Few mutants of heat shock genes in Drosophila are described, presumably owing to the absence of a simple screen for such a mutation. To our knowledge, *Hsp28*^{stl} is the first developmental mutation to be described at the 67B locus; the differential effects of the mutation provide insight into the mechanism of *Hsp28* regulation.

The results obtained suggest that in the mutant *Hsp28*^{stl}, heat-induced expression of *Hsp28* is regulated by sequences moved distally due to the insertion of the transposable element. RNA polymerase prob-

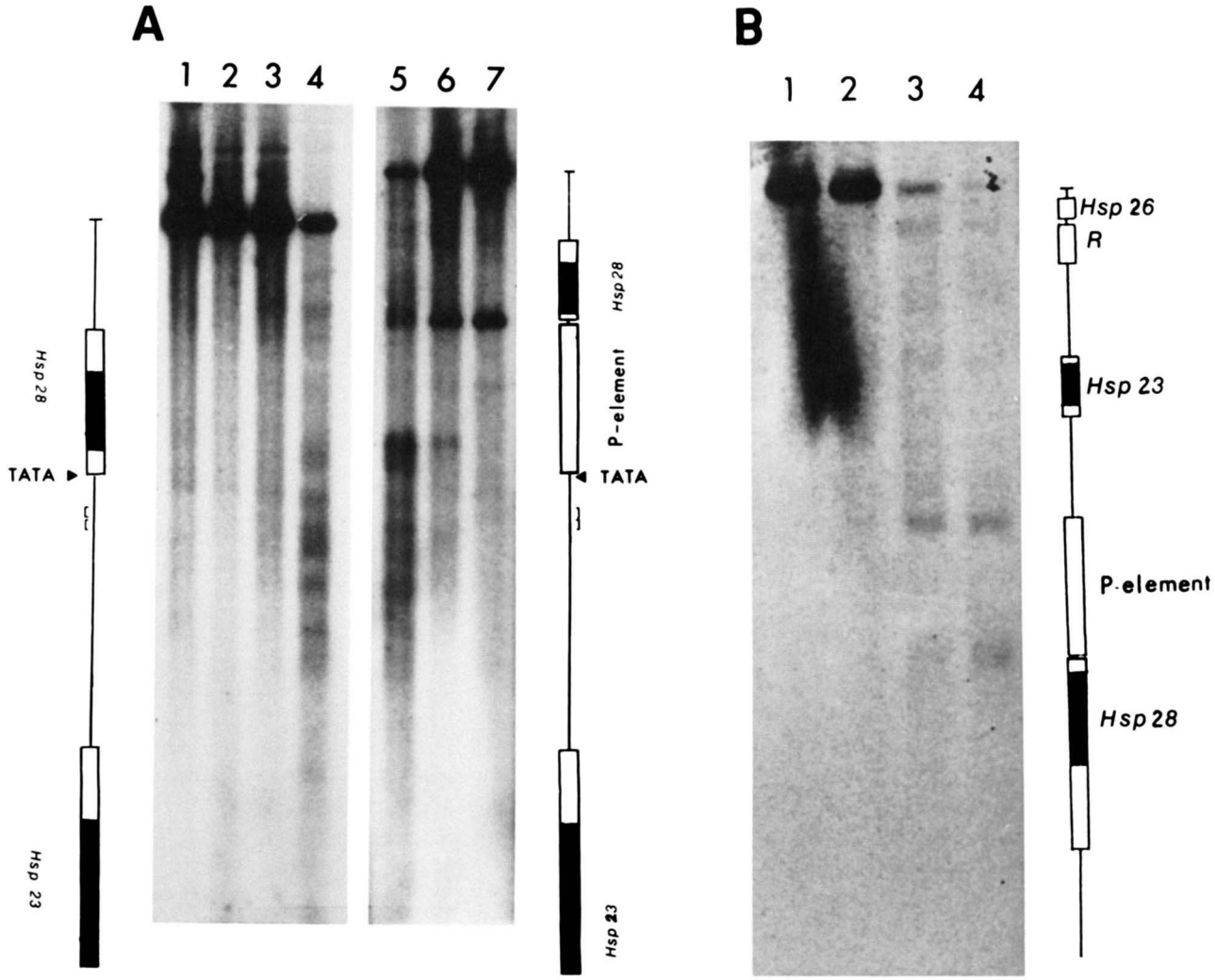

FIGURE 6.—The chromatin structure of *Hsp28*^{stl} in third instar larvae. A, Nuclei were prepared from third instar Oregon R and *Hsp28*^{stl} non-heat-shock larvae and aliquots of nuclei were digested to various extents with DNase I. The purified nuclear DNA was digested to completion with *Eco*RI, electrophoresed in a 1.2% agarose-TAE gel, and subjected to indirect end labeling analysis using 88.2 as a hybridization probe (CORCES *et al.* 1980). Lane 1: Oregon R, control (no DNase I); lanes 2–4: Oregon R, digested with increasing amounts of DNase I; lanes 6 and 5: *Hsp28*^{stl}, digested with increasing amounts of DNase I; lane 7; *Hsp28*^{stl}, control (no DNase I). Maps flanking each panel indicate the relative positions of sequence elements within the *Eco*RI fragment under analysis. The small square brackets in each map indicate the relative positions of sequences showing homology to the heat shock consensus sequence (see DISCUSSION). An open box in the right-hand map indicates the relative position of the defective *P*-element insert. B, Nuclei from *Hsp28*^{stl} larvae were digested with DNase I, and the DNA analyzed as in panel A, except that the restriction enzyme was *Bam*HI, samples were electrophoresed in a 0.8% agarose-TAE gel, and the indirect end-labeling probe was fragment a (Figure 1). Lane 1: control (no DNase I); lanes 2–4: increasing amounts of DNase I.

ably associates with the normal TATA sequence, which in *Hsp28*^{stl} is distal to the *P*-element insert, initiating transcription at some nearby point downstream and within the *P*-element. This would give rise to the low level fusion transcript, 1.3 kb larger than normal *Hsp28*, detected in Northern blots of *Hsp28*^{stl} heat shock RNA. This interpretation is consistent with the results of deletion analysis. From a series of engineered deletions transduced into flies using *P*-element germ line transformation, HOFFMAN and CORCES (1986) reported that deletion of sequences more than 124 bp upstream of the *Hsp28* cap site abolishes all detectable transduced *Hsp28* heat-shock-induced expression. Using a transient expression assay of *Hsp28* upstream sequences, RIDDIHOUGH and PELHAM (1986) further localized sequences essential to heat induction to the interval −455 to −227. Finally, CARTWRIGHT and ELGIN (1986) report a major shift in nuclease sensitivity (from a hypersensitive site to strong nuclease protection) upon heat shock centered at position −310. This region contains three blocks of sequences with an excellent match of nondegenerate positions to the heat shock consensus sequence (MIRAULT, SOUTHGATE and DELWART 1982; PELHAM 1982; RIDDIHOUGH and PELHAM 1986). In nuclei of *Hsp28*^{stl} flies, the chromatin structure of this region, now shifted 1.3 kb away from the *Hsp28* coding sequence, is not visibly altered by the *P*-element insertion (Figure 6A). One can suggest that the interaction of the heat shock transcription factor with this region

can stimulate the initiation of transcription, resulting in the induced product seen.

Initiation of transcription does appear to occur, however, at or close to the normal start site in adult fertile *Hsp28*^{stl} females. To account for this, we suggest that at least some of the sequence elements directing ovarian expression are clearly separable from the heat shock regulatory elements and may reside downstream of the insert. HOFFMAN and CORCES (1986) similarly observed approximately 30% of wild-type expression in non-heat-shocked prepupae and fertile females in cases where sequences upstream of −124 bp had been deleted and no heat-shock-induced expression was observed. Note, however, that no *Hsp28* RNA was detected of any size in *Hsp28*^{stl} white prepupae. Prepupal regulation therefore seems to differ to some degree from ovarian regulation. Differences in mRNA stability, as well as differences in transcription, could play a role in maintaining the levels of developmentally regulated *Hsp28* expression. Preferential RNA stabilization is known to play an important role in ecdysone-regulated expression of the small heat shock genes (VITEK and BERGER 1984).

Sequence analysis of the cloned *Hsp28*^{stl} allele places the site of the *P*-element insertion between the *Hsp28* "TATA box" and the normal *Hsp28* mRNA cap site sequence. This region is hypersensitive to several DNA cleavage reagents in nuclei from embryos and larvae, as well as from tissue culture cells (COSTLOW and LIS 1984; CARTWRIGHT and ELGIN 1986). The relatively exposed state of the DNA in this region in nuclei might render it particularly sensitive to transposable element insertions. While this speculation makes the untested assumption of hypersensitive sites in germ line chromatin, we note that in 0–2-hr-old *Drosophila* embryos (*i.e.*, preblastoderm), DNase I hypersensitive sites are already established upstream of *Hsp28*, before *Hsp28* expression can be heat induced (LOWENHAUPT *et al.* 1983).

Another notable feature of the defective *P*-element at *Hsp28*^{stl} is the fact that only two nucleotides of the target site are duplicated at each end of the insert. This is the first instance that we are aware of of an exception to the finding that *P*-element insertion is accompanied by an 8-bp duplication of the target site (O'HARE and RUBIN 1983). We cannot rule out the possibility that the original insertion was flanked by 8-bp duplications, but that a 6-bp deletion occurred subsequently; we simply call attention to this apparent exception to the 8-bp rule since, if similar exceptions are reported, such exceptions may have implications for models of *P*-element transposition.

Both ends of the defective *P*-element at *Hsp28*^{stl} appear to be hypersensitive to DNase I (Figure 6B). While a majority of the normal *P*-element sequences are deleted in this defective copy, the 31-bp terminal repeats necessary for transposition remain intact. We have recently shown that the termini of the *P*-element-derived vector Carnegie 4 (RUBIN and SPRADLING 1983) are also DNase I hypersensitive sites in chromatin (EISSENBERG and ELGIN, 1986). A previous report showed that the termini of the *copia*-like transposable element HMS Beagle contained DNase I hypersensitive sites as well (EISSENBERG *et al.* 1984), suggesting that this may be a general feature of transposable elements.

The presence of intact terminal repeats flanking the defective *P*-element at 67B in *Hsp28*^{stl} suggests the possibility that, in the presence of *P*-element transposase function, this element could be mobilized to generate further regulatory mutations at this locus. Since imprecise transposable element excision can sometimes lead to polar chromosomal deletions, *Hsp28*^{stl} may also be useful in generating deletions within the 67B locus, which in turn could lead to a better understanding of the function of small heat shock genes in the physiology of the stress response in Drosophila.

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