DOI: 10.1534/genetics.105.053959

Remarkable Site Specificity of Local Transposition Into the *Hsp70* Promoter of *Drosophila melanogaster*

Victoria Y. Shilova,* David G. Garbuz,* Elena N. Myasyankina,* Bing Chen,† Michael B. Evgen'ev,*,‡ Martin E. Feder†,§,[1] and Olga G. Zatsepina*

**Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 117984 Moscow, Russia, ‡Institute of Cell Biophysics, 142290 Puschino, Russia, †Department of Organismal Biology and Anatomy and §The Committees on Evolutionary Biology, Genetics, and Molecular Medicine, The University of Chicago, Chicago, Illinois 60637*

Manuscript received November 25, 2005
Accepted for publication March 19, 2006

ABSTRACT

Heat-shock genes have numerous features that ought to predispose them to insertional mutagenesis via transposition. To elucidate the evolvability of heat-shock genes via transposition, we have exploited a local transposition technique and *Drosophila melanogaster* strains with *EPgy2* insertions near the *Hsp70* gene cluster at 87A7 to produce numerous novel *EPgy2* insertions into these *Hsp70* genes. More than 50% of 45 independent insertions were made into two adjacent nucleotides in the proximal promoter at positions −96 and −97, and no insertions were into a coding or 3′-flanking sequence. All inserted transposons were in inverse orientation to the starting transposon. The frequent insertion into nucleotides −96 and −97 is consistent with the DNase hypersensitivity, absence of nucleosomes, flanking GAGA-factor-binding sites, and nucleotide sequence of this region. These experimental insertions recapitulated many of the phenotypes of natural transposition into *Hsp70*: reduced mRNA expression, less Hsp70 protein, and decreased inducible thermotolerance. The results suggest that the distinctive features of heat-shock promoters, which underlie the massive and rapid expression of heat-shock genes upon heat shock, also are a source of evolutionary variation on which natural selection can act.

MOBILIZATION of DNA is a principal source of genetic and genomic variation (Kazazian 2004). Except for a few examples, accumulation of mobile elements accounts for 22 and 50% of the Drosophila and human genomes, respectively (Kapitonov and Jurka 2003; Bellen *et al.* 2004), and the proliferation of one class of mobile elements is proposed to account for the extraordinary gene duplication that accompanied the divergence of humans from sister groups (Bailey *et al.* 2003). Mobilization of DNA, moreover, is not solely a past event, but is ongoing in natural populations, where it may underlie evolutionary adaptation. In Drosophila, for example, the insertion of transposable elements into proximal promoters of heat-shock and cytochrome P450 genes, which thereafter segregate as alleles in natural populations, underlies adaptation to temperature and insecticides, respectively (Michalak *et al.* 2001; Bettencourt *et al.* 2002; Daborn *et al.* 2002; McCollum *et al.* 2002; Lerman *et al.* 2003; Petrov *et al.* 2003; Schlenke and Begun 2004; Aminetzach *et al.* 2005; Bogwitz *et al.* 2005; Lerman and Feder 2005; Marsano *et al.* 2005). Importantly, transposition into genes is not random, but requires insertion sites that are physically accessible to the transposition machinery, and all genes (or regions of genes) are not equal in providing access (Bellen *et al.* 2004). Presently, however, the exact basis for transposition into specific nucleotides and the avoidance of others is unknown (Bellen *et al.* 2004), and understanding this basis would enable prediction of the evolvability of specific sites via transposition.

To address this issue, we have exploited a variant of local transposition, which Timakov *et al.* (2002) have used to mutagenize specific regions of the Drosophila genome. Its principle is that *P* elements insert preferentially in genes near the starting site, and hence judicious choice of a starting *P* element can yield numerous transpositions into a target gene (if, that is, the target is susceptible to transposon insertion). As a primary target, we have selected the *Hsp70* gene cluster at 87A7. The BDGP Gene Disruption Project (Bellen *et al.* 2004) has now yielded strains with ideal starting transposons distributed throughout the euchromatin, and we have selected two strains with *EP* transposons in neighboring genes of the target.

The 87A7 *Hsp70* cluster contains two (*Hsp70Aa* and *Hsp70Ab*) of the five to six nearly identical genes that encode Hsp70, the principal heat-inducible molecular chaperone in *Drosophila melanogaster* (Bettencourt and Feder 2002). Several features of the cluster and its genes may predispose it to transpositional mutagenesis. The region upstream of the *Hsp70* TATA element

[1]*Corresponding author:* Department of Organismal Biology and Anatomy, University of Chicago, 1027 E. 57th St., Chicago, IL 60637. E-mail: m-feder@uchicago.edu

contains multiple binding sites for the sequence-specific regulatory proteins, GAGA factor (GAF) and heat-shock factor (HSF). Prior to heat shock, GAF resides on the *Hsp70* promoter (Gilmour *et al.* 1989; O'Brien *et al.* 1995). The binding of GAF appears to maintain the promoter region in a nucleosome-free conformation (Karpov *et al.* 1984; Udvardy *et al.* 1985; Nacheva *et al.* 1989; Becker and Craig 1994; Tsukiyama *et al.* 1994; Georgel 2005), as is consistent with the DNase hypersensitivity of the region (Weber *et al.* 1997). The polymerase apparatus is preassembled but stalled, awaiting the arrival of activated HSF. Thus, the *Hsp70* promoter is "bookmarked" (Xing *et al.* 2005). These features are thought to allow ready access of the general transcription factors to the core promoter and thus facilitate rapid induction of transcription upon heat shock, but may thereby facilitate the access of the transposition machinery to the underlying DNA, especially at or near the DNase hypersensitive sites. Finally, although nominally a heat-shock gene, *Hsp70* is transcribed in the male germline (Boutanaev *et al.* 2002; Lakhotia and Prasanth 2002), which should to facilitate germline transposition. Indeed, numerous transposons have naturally and independently inserted into the *Hsp70* proximal promoter (Michalak *et al.* 2001; Zatsepina *et al.* 2001; Bettencourt *et al.* 2002; Lerman *et al.* 2003; Lerman and Feder 2005; our unpublished data).

Insertional mutagenesis can have a dramatic impact on gene expression by adding novel regulatory elements to a gene or by disrupting preexisting regulatory regions (Kazazian 2004; Puig *et al.* 2004; Lerman and Feder 2005). In *Hsp70*, four heat-shock elements (HSEs) bind activated HSF; their number and spacing is crucial for full-strength gene expression (O'Brien and Lis 1991; Lis and Wu 1993; Amin *et al.* 1994; Li *et al.* 1996; Mason and Lis 1997; Weber *et al.* 1997). In natural populations, insertion of transposons into the *Hsp70* promoter disrupts this spacing and thereby reduces *Hsp70* expression (Lerman *et al.* 2003; Lerman and Feder 2005). This impact is due solely to the physical disruption of the promoter and not to transposition of regulatory elements, as replacement of the transposons with random DNA sequences of identical size has the same phenotype (Lerman and Feder 2005). In turn, reduced *Hsp70* transcription can result in reduced (or, paradoxically, increased) Hsp70 protein, which affects inducible thermotolerance, growth, and development (Lerman *et al.* 2003). Thus, experimental mutagenesis of *Hsp70* via local transposition may illustrate molecular and organismal phenotypes as well as the details of target sites.

Here we report outcomes of a local transposition experiment in which a mobilized transposon has inserted into one or both of the *Hsp70* genes at 87A7 in 45 independent trials. Remarkably, >50% of these insertions were into two adjacent nucleotides in the proximal promoter at positions −96 and −97, and no insertions were into a coding or 3′-flanking sequence. DNase hypersensitivity, absence of nucleosomes, flanking GAGA-factor-binding sites, and preferred nucleotide sequence all coincide at −96 and −97. These experimental insertions, moreover, recapitulated many of the phenotypes of natural transposition into *Hsp70*, further supporting the hypothesis that heat-shock genes are exceptionally prone to evolution via transposition (Lerman *et al.* 2003; Lerman and Feder 2005).

MATERIALS AND METHODS

Drosophila strains: All flies were reared on a yeast, cornmeal, molasses, and agar medium at 25°. A transposase source stock (w[*]; ry[506] Sb[1] P{ry[+t7.2] = Delta2-3}99B/TM6B, Tb[1]), hereafter "Delta2-3," and two stocks with insertions near the 87A7 *Hsp70* gene cluster were obtained from the Bloomington Drosophila Stock Center. The first insertion stock (stock no. 15616), hereafter named "US-4," had the following genotype: y[1] w[67c23]; P{w[+mC] y[+mDint2] = *EPgy2*} EY03020; the second stock, hereafter named "US-2" (stock no. 15904), had the following genotype: y[1] w[67c23]; P{w[+mC] y[+mDint2] = *EPgy2*} EY03490. US-4 contains an *EPgy2* insertion at +243 nucleotides relative to the transcription start of gene CG12213, and US-2 contains the same element at +33 nucleotides relative to the transcription start of *aurora* (*aur*). Figure 1 depicts the orientations of these insertions and of the host and *Hsp70A* genes. The insertion in US-4 is located ~8 kb from the 5′-end of *Hsp70Ab*, and that in US-2 is located ~5 kb from the 5′-end of *Hsp70Ab*.

Genetic screen to detect local insertions: Our isolation of local reinsertions of the *EPgy2* element followed the approach of Golic (1994) and Timakov *et al.* (2002). This approach rests on two assumptions: that the starting element used to produce local insertions is retained in local transposition and that the expression of the marker (*white*) gene in the *P*-element construct would identify flies with additional insertions by darker eye pigmentation. For phenotype studies, selected strains carrying one or two insertions in the *Hsp70A* genes were made homozygous by individual crossings of flies with darker eyes. These crosses yielded the following homozygous (nonsegregating) strains: 1IIa, 5IId, 30IIb, 111IIa, 123Ia, 134Ib, 186Ib, 246I, 253Ia, 310II, 332II, 369I, and α34I (Table 1).

DNA manipulations and Southern analysis: Isolation of genomic DNA from adult flies and Southern blot analysis to detect new insertions in *Hsp70* genes was performed according to Evgen'ev *et al.* (2004). Five micrograms of each DNA sample was digested with *Hin*dIII and *Bam*HI restriction endonucleases. After agarose gel electrophoresis, the gel was treated for 15 min in 0.25 M HCl and then incubated twice in denaturing buffer (1.5 M NaCl/0.5 M NaOH) for 30 min. After a 30-min incubation in neutralization buffer, gels were capillary blotted onto nylon membranes and fixed by UV crosslinking using the UV Stratalinker 2400 (Stratagene, La Jolla, CA) protocol. Standard high-stringency hybridization and wash conditions were used for Southern blot analysis. To detect the positions of *Hsp70* sequences in Southern blots, fragments of cloned *D. melanogaster Hsp70* genes were labeled by random priming and used as probes. The original screen of Southern blots was with 5′-specific and 3′-specific probes prepared from the *Cla*I–*Sal*I fragment of *D. melanogaster Hsp70* (McGarry and Lindquist 1985), where the 5′-specific probe is the *Cla*I–*Bam*HI fraction and the 3′-specific probe is the *Bam*HI–*Sal*I fraction.

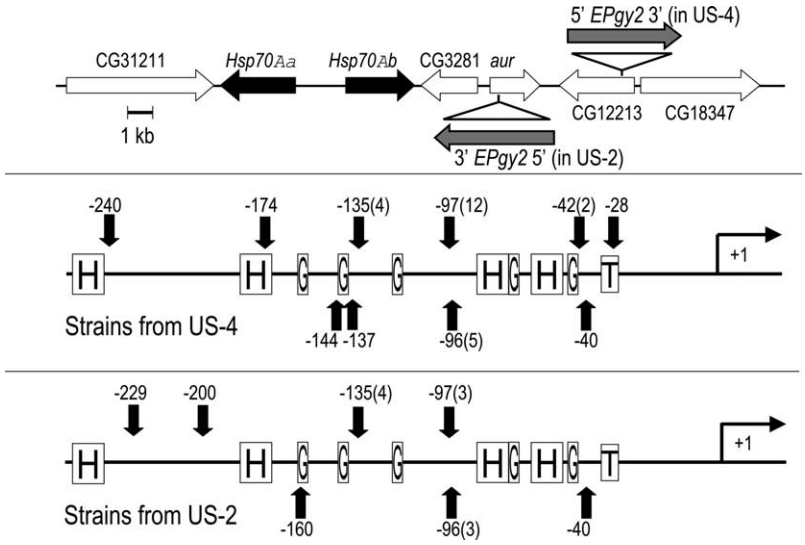

FIGURE 1.—Starting and insertion sites for local transposition. (Top) Region 87A of chromosome 3, indicating position and orientation of the two *Hsp70* genes in this region and adjacent genes, as well as approximate starting positions and orientations of the *EPgy2* transposons to be mobilized. (Middle) For *Hsp70Aa* and *Hsp70Ab* combined, insertion sites of the *EPgy2* transposon originally located in CG12213 (US-4 strain). Numbers before parentheses refer to nucleotide position relative to transcription start; numbers of independent transpositions into the indicated nucleotide are in parentheses. Two additional insertions were detectable but could not be localized. Approximate positions of heat-shock (H) and GAGA (G) elements and the TATA box are indicated. Of these 29 transpositions, 87% were into *Hsp70Aa*, and all were in an orientation opposite to that of the starting transposon. (Bottom) For *Hsp70Aa* and *Hsp70Ab* combined, insertion sites of the *EPgy2* transposon originally located in *aur* (US-2 strain). Data are plotted as in the middle. Of these 14 transpositions, 57% were into *Hsp70Aa*, and all were in an orientation opposite to that of the starting transposon.

Detection of local insertions with PCR: Drosophila genomic DNA was isolated as described (EVGEN'EV *et al.* 2004). Each polymerase chain reaction consisted of 1.25 units of Taq DNA polymerase (Promega, Madison, WI) per probe, 0.1 μg DNA in 30 μl of buffer (Promega), 1.5–2 μM $MgCl_2$, 0.2 mM of each dNTP, and 5 pM of each primer. Reaction conditions for all PCR reactions included 1.5 min at 94°; 30 cycles of 94° for 1 min, 56°–64° for 30 sec, and 72° for 2 min; and 72° for 5 min in a PTC-100 thermocycler (MJ Research, Watertown, MA). Primers were complementary to the *EPgy2* construct (TIMAKOV *et al.* 2002), *Hsp70A* (GenBank accession no. AE003693), and adjacent genes. Figure 2 indicates their position and Table 2 their sequence.

Sequencing: Sequences flanking *EPgy2* insertions were obtained via PCR with a genomic primer (2, 53, 54, or 55, Table 2) and a primer specific to the 5′-end or 3′-end of *EPgy2*. The PCR product was loaded onto a 1% agarose gel in TAE, and the band containing the amplified DNA was excised and purified with a QIAquick PCR purification kit (QIAGEN, Chatsworth, CA) for subsequent sequencing with one of the primers used in the PCR reaction. The PCR fragments were sequenced. Sequencing was with Sequenase (Amersham, Buckinghamshire, UK) and ABI 377 sequencers. In some cases internal sequencing primers were used to provide double-strand coverage. Sequences were assembled manually and aligned using CLUSTAL X (JEANMOUGIN *et al.* 1998).

Quantitative RT–PCR: Equal amounts (1 μg) of RNA were used to synthesize first-strand cDNA with oligo(dT) in the reverse transcription reaction [Abgene (Rochester, NY) first-strand synthesis kit] according to the manufacturer's

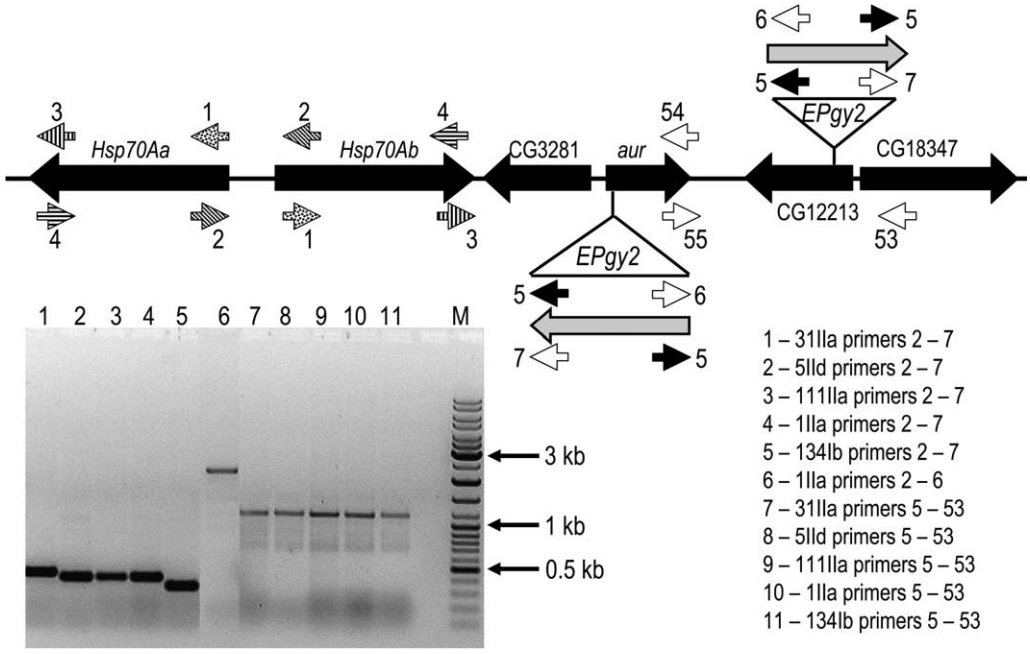

FIGURE 2.—Determination of location and orientation of *EPgy2* constructs via the polymerase chain reaction. (Top) Sites complementary to PCR primers described in MATERIALS AND METHODS. (Bottom) Amplicon size, indicating differing sites of *EPgy2* transposition into *Hsp70Aa* (lanes 1–5) and the unchanged location of the starting transposon (lanes 7–11) in the specified lines.

instructions. *Hsp70A* mRNA was determined by real-time quantitative PCR with an MJ Opticon detector, SYBR green detection method, and ABgene reagents. The housekeeping gene *rp49* was used as a loading control. All reactions were performed in triplicate. The copy numbers of RNA encoded by the *Hsp70Aa* and *Hsp70Ab* genes, collectively, were standardized against *rp49* mRNA in each sample. The PCR reaction used primers (Table 2) complementary to both *Hsp70Aa* and *Hsp70Ab* but not to the *Hsp70* genes at 87C1. Specificity was established in preliminary experiments with the *Hsp70A* and *Hsp70B* deletion strains (Gong and Golic 2004) (data not shown).

Thermotolerance studies: Procedures were identical to those of Garbuz *et al.* (2002). Eclosing individuals were sequestered daily and, when 4 days old, were transferred by aspiration to a preheated polypropylene vial, which was immersed in a thermostated water bath for 30 min. Each vial usually contained 75–100 animals. To determine basal thermotolerance, vials were placed at one of a series of temperatures ranging from 38.5° to 40°. To determine inducible thermotolerance, similar determinations ensued after flies first underwent pretreatment at 35° for 30 min and 25° for 1 hr. Tolerance was assessed as the proportion of flies that could walk 48 hr after heat shock.

Immunoblotting: Procedures were identical to those of Garbuz *et al.* (2002). In brief, lysates were prepared from 4-day-old flies after heat shock at various temperatures. After sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of 10 μg total protein per lane, the proteins were transferred to a nitrocellulose membrane (Hybond ECL; Amersham) according the manufacturer's protocol. Monoclonal antibody specific to *D. melanogaster* Hsp70 (7FB) was obtained from Susan Lindquist (University of Chicago). Immune complexes were detected via chemoluminescence (ECL kit, Amersham) and 3,3′-diaminobenzidine (Sigma, St. Louis) with appropriate peroxidase-conjugated anti-rat secondary antibodies.

Preparation of RNA and Northern hybridization: Procedures were identical to those of Garbuz *et al.* (2003). RNA from adult flies was prepared by the standard method with guanidine isothiocyanate, separated by gel electrophoresis, and transferred to a membrane for hybridization with the *Cla*I–*Bam*HI fragment of the *D. melanogaster Hsp70* gene (McGarry and Lindquist 1985). Hybridization was overnight at 42° in 50% formamide, followed by two 20-min washes in 2× SSC, 0.2% SDS at 42°, and one 20-min wash in 0.2× SSC, 0.2% SDS at 68°.

RESULTS

Producing local insertions in the 87A region of chromosome 3: The *EPgy2* construct contains *mini-white*, whose phenotype is a light-orange eye color, as a marker. In progeny of crosses of Delta2-3 with flies bearing this construct in either of the starting locations, the element was frequently excised, as was evident from patches of white cells (mosaics) on a background of cells with light-orange pigmentation (data not shown).

Females of both stocks with starting elements were crossed *en masse* to Delta2-3 males and resulting F_0 males were crossed singly to *yw/yw* females (Df1 strain). F_1 males exhibiting darker eye color (dark orange or light red) in comparison with other males were crossed with *yw/yw* females individually and checked for segregation in the subsequent generations. In the F_1 for US-4, 562 males of 7400 exhibited darker color. Therefore, frequency of transposition was ~7.5%. In the US-2 series, 232 of 4725 F_1 males carried new insertions; thus, total transposition frequency for this series was 4.9%. Segregating strains displaying various eye colors in the progeny of individual crosses of these males probably resulted from nonlocal transpositions and were not analyzed further.

Strategy and proof of principle: All nonsegregating F_1 strains with dark eye color first underwent screening by Southern blot analysis to determine into which, if any, of the six *Hsp70* genes *EPgy2* had inserted. In this analysis, *Hin*dIII–*Bam*HI restriction digests yield products readily assignable to each *Hsp70* gene (Figure 3). Because the *EPgy2* construct includes a *Hin*dIII site, the insertion of the element in the heterozygous condition should yield an additional band of lower molecular weight (Figure 3), while in homozygous condition it should eliminate the band corresponding to the *Hsp70* gene(s) into which each insertion was made (Figure 4). The Southern blots were hybridized with either 5′- or 3′-specific *Hsp70* probes to localize insertions within the *Hsp70* genes. Hybridization with the 3′ probe detected no insertions of *EPgy2* in the 3′-coding part of the gene or its flanking region. Reprobing of the same blots with the 5′ probe, by contrast, detected numerous heterozygous insertions in this region (Figure 3). Crosses of these strains yielded homozygous strains (Figure 4) in many but not in all cases, probably because the insertions were sometimes associated with lethality or sterility. The specific site of insertion was determined by PCR and subsequent sequence analysis (see below). Several insertions (three for US-4 and three for US-2) in *Hsp70* genes apparently did not contain the *EPgy2* construct (Figure 4). The high molecular weight bands in lanes 12–15 and 17–18 (strains 2Ia, 99IIa, 244Ia, α3II, α120I, and α121I, Figure 4) may be due to mutations in the restriction site and/or inserting element or to the insertion of mobile elements other than the *EPgy2* construct (see Lewis and Brookfield 1987).

Localization of insertions in *Hsp70* genes: Southern blot analysis (*e.g.*, Figures 3 and 4) of the *Hsp70Aa-Hsp70Ab* gene cluster at 87A7 detected 31 independent insertions of the *EPgy2* construct from US-4 and 14 insertions from US-2 among 375 and 160 nonsegregating strains, respectively. Hybridization with *Hsp70* 5′-specific and 3′-specific probes revealed that all of the insertions were into the 5′ regions of *Hsp70* genes. A combination of PCR and sequencing resolved the specific insertion sites (Table 1; Figures 1 and 2). Remarkably, 55% of the US-4 insertions and 40% of the US-2 insertions were into the same two nucleotides in the *Hsp70A* promoters, positions −96 and −97. Each insertion was a singleton except for cross 30IIb (derived from US-4) and cross α198I (derived from US-2), in which both *Hsp70Aa* and *Hsp70Ab* received insertions. In two cases (288Ia-k and 332II-332IIb) insertions in the

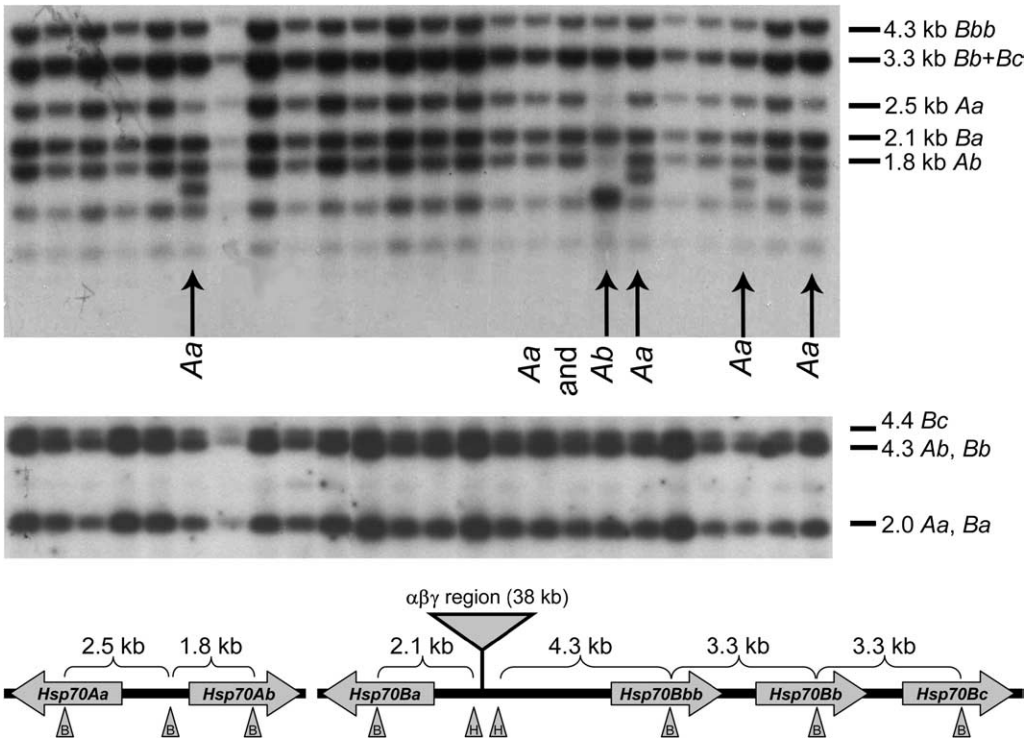

FIGURE 3.—Representative genomic Southern blot used to screen nonsegregating lines for transposition into *Hsp70* genes: lines heterozygous for insertions. (Top) Hybridization with the *Cla*I–*Bam*HI fragment of *Hsp70*, which is specific for the 5′ region of *Hsp70* genes. *Bam*HI–*Hin*dIII digestion yields fragments of indicated sizes. As indicated by arrows, in strains heterozygous for *EPgy2* elements in *Hsp70* genes, a *Hin*dIII site in *EPgy2* reduces the concentration of the band corresponding to the corresponding gene and results in a band of lower molecular weight. (Middle) Hybridization with the *Bam*HI–*Sal*I fragment of *Hsp70*, which is specific for the 3′ region of *Hsp70* genes. *Bam*HI–*Hin*dIII digestion yields fragments of indicated sizes. As indicated by the absence of variation from canonical fragment size, no *EPgy2* elements inserted into the 3′ portion of *Hsp70* genes. (Bottom) Organization of the Drosophila *Hsp70* genes in the experimental lines (GONG and GOLIC 2004), indicating the *Bam*HI–*Hin*dIII restriction sites (B and H in triangles, respectively) and origin of the corresponding fragments in the top of the figure. The first two genes are at 87A7, and the last four genes are at 87C1. Not to scale.

progeny of US-4 strain F_1 males were identical, consistent with premeiotic transposition.

In ~300 strains with insertions outside of *Hsp70A*, insertions were mapped to specific genes with the 53-5, 54-5, 55-5, and 3-5 primer pairs (Table 2). Of these, 4% were in *aurora*, CG18374, and CG3281, genes near *Hsp70A* (Figure 2). In contrast to the insertions in *Hsp70A*, the insertions in these genes were not concentrated in promoter regions but were randomly distributed along the genes' length.

The orientation of the local insertions relative to the starting element was determined with primers specific for the putative host gene and primers complementary to the *P*-element-containing construct. All insertions into *Hsp70A* were oriented opposite to the starting element (Figure 1), implying that constructs always underwent a 180° rotation during insertion.

Interestingly, in crosses involving the US-4 strain, most (87%) insertions were into *Hsp70Aa*, the gene more distal from the starting position. By contrast, in the crosses involving US-2, insertions were more evenly distributed (57% in *Hsp70Aa* and 43% in *Hsp70Ab*).

Because of the extraordinary conservation of *Hsp70* coding sequence, the same primers used to detect

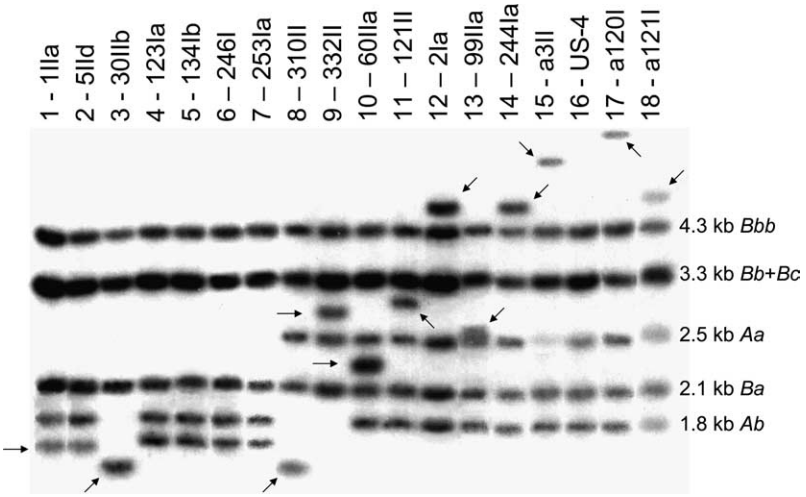

FIGURE 4.—Representative genomic Southern blot used to screen nonsegregating lines for transposition into *Hsp70* genes: lines homozygous for insertions and unusual mutants. Lanes 1–8: insertions of the *EPgy2* element only; see Table 1 for specific insertion sites. Lane 16: a strain with the *EPgy2* element in the starting position. Lane 9: 1.5 kb of GC12213 flanking sequence has been cointegrated with the P-construct. Lane 10: 1 kb of GC12213 flanking sequence has been cointegrated with the P-construct. Lane 11: ~2.3 kb of GC12213 flanking sequence has been cointegrated with the P-construct. Lanes 12–15, 17, and 18: instances of aberrant Southern analyses but with no *EPgy2* insertion into the *Hsp70A* locus detectable by PCR. These may be due either to aberrations or to insertions of unidentified transposons.

TABLE 1

Strains harboring local transpositions into *Hsp70Aa*, *Hsp70Ab*, and neighboring genes

Strains derived from US-4

	Strain:	1IIa	2Ia	5IId	30IIb	31IIa	48Ia	60IIa[a]	61Ib	86I	99IIa
2-5 primers		402		402	401	440	401	1.2 kb	401	402	
2-7 primers		+		+		+	+	+	+	+	
2-6 primers					+						
Localization		−97	Not P	−97	−96	−135	−96	−240	−96	−97	Not P
Gene		*Aa*		*Aa*	*Aa Ab*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	

	Strain:	105Ig	111IIa	121II	123Ia	134Ib	163Ia	169Ig	177I	179I	184I
2-5 primers		401	402	2.6 kb	440	332	402	402	401	402	1.6 kb
2-7 primers		+	+	+	+	+	+	+	+	+	+
2-6 primers											
Localization		−96	−97	Not detected	−135	−28	−97	−97	−96	−97	Not detected
Gene		*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*

	Strain:	186Ib	244Ia	246I	253Ia	253Ib	258II	284Ia	288Ia	288Ib	288Ic
2-5 primers		346		440	402	402	440	402	344	344	344
2-7 primers		+		+	+	+	+	+	+	+	+
2-6 primers											
Localization		−42	Not P	−135	−97	−97	−135	−97	−40	−40	−40
Gene		*Aa*		*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*	*Aa*

	Strain:	288Id	288Ie	288Ik	295II	309Ib	310II	332II[a]	332IIb[a]	369I	377II
2-5 primers		344	344	344	402	346	442	2kb	2kb	449	479
2-7 primers		+	+	+	+					+	
2-6 primers						+	+	+	+		+
Localization		−40	−40	−40	−97	−42	−137	−97	−97	−144	−174
Gene		*Aa*	*Aa*	*Aa*	*Aa*	*Ab*	*Ab*	*Ab*	*Ab*	*Aa*	*Ab*

Strains derived from US-2

	Strain:	α3II	α9Ib	α21Ib[a]	α33Ib	α34I	α79Ia	α36I	α196Ia	α200I	α198I
2-5 primers			402	726	505	402	401		440	440	401
2-7 primers						+	+			+	+
2-6 primers			+	+	+				+		
Localization		Not P	−97	−229	−200	−97	−96	+791	−135	−135	−96
Gene			*Aa*	*Aa*	*Aa*	*Ab*	*Ab*	CG3281	*Aa*	*Ab*	*Ab*

	Strain:	α198I	α250Ia	α138IIa	148IIa	α196II	α59Ib	α199II	α227I	α120I	α121I
2-5 primers		465	440	402	401	344			440		
2-7 primers						+			+		
2-6 primers		+	+	+	+						
Localization		−160	−135	−97	−96	−40	+103	+252	−135	Not P	Not P
Gene		*Aa*	*Aa*	*Aa*	*Aa*	*Ab*	CG3281	CG3281	*Ab*		

"Localization" refers to the relative position of the insertion to the transcription start. "Not P" refers to the insertions of other unknown TEs. "+" indicates positive PCR with 2 – 6 or 2 – 7 primers. Insertion in α 36I was detected with primers 3 and 6 and in α 59Ib and α199II with primers 6 and 7. The total number of DNAs isolated from the nonsegregating strains using the first construct (US-4) is 380. The total number of DNAs isolated from the nonsegregating strains using the second construct (US-2) is 100.

[a] Long fragments with part of CG12213 or *aur*.

transposition into the 87A7 *Hsp70* cluster were sufficient to detect transposition into the 87C1 *Hsp70* cluster, as were the Southern blots. Neither screen detected any transposition into the 87C1 cluster, which is much more distant from the starting transposons than is 87A7.

Gene expression and protein levels: Gene-cluster-specific quantitative RT–PCR (Q-RT–PCR) primers enabled distinction of *Hsp70A*-specific mRNA from total *Hsp70* mRNA. In four of five cases, local transposition into the *Hsp70A* gene cluster reduced *Hsp70A* mRNA

TABLE 2

Primers used for this work

Primer	Sequence	Context
1	5′-ATGCCTGCTATTGGAATCGATC-3′	5′ *Hsp70* inward
2	5′-GATCGATTCCAATAGCAGGCAT-3′	5′ *Hsp70* outward
3	5′-GAGGAGTTCGACCACAAGC-3′	3′ *Hsp70* outward
4	5′-GCTTGTGGTCGAACTCCTC-3′	3′ *Hsp70* inward
5	5′-CGACGGGACCACCTTATGTTA-3′	Invert repeat of *P* element
6	5′-AATTCGTCCGCACACAAC-3′	5′ *P* element outward
7	5′-ATCATATCGCTGTCTCACTCAG-3′	3′ *P* element outward
53	5′-TACACGAAGGTGCAAATCG-3′	To CG18347
54	5′-GTCCCGAATTAGCAGTAATC-3′	To 3′ of *aur* inward
55	5′-GATTACTGCTAATTCGGGAC-3′	To 3′ of *aur* outward
Hsp70A-1	5′-CCTGGAGAGCTACGTCTTCAAT-3′	Used in Q-RT–PCR
Hsp70A-2	5′-GTCGTTGCACTTGTCCAA-3′	Used in Q-RT–PCR
rp49-1	5′-CGCACCAAGCACTTCATCC-3′	Used in Q-RT–PCR
rp49-2	5′-AGCGGCGACGCACTCTGT-3′	Used in Q-RT–PCR

See also Figure 2.

present after a 37.5° heat shock for 30 min (Figure 5). The reduction was similar for insertions at −135, −97 (strain 111IIa), and −28 in *Hsp70Aa* and at −96 in both *Hsp70Aa* and *Hsp70Ab* and was 20–40% of levels in the US-4 strain from which these strains were derived (Figure 5, left). In the pair of these strains examined after a 37° heat shock for 30 min, total *Hsp70* mRNA (*i.e.*, *Hsp70A* mRNA + *Hsp70B* mRNA) was 75–80% of levels in the US-4 strain. Twelve hours after heat shock, total *Hsp70* mRNA was 30–40% of levels in the US-4 strain.

By contrast, in an independently derived strain with a transposon insert at −97 in *Hsp70Aa* (strain 1IIa), *Hsp70A* mRNA was the same as in the US-4 strain from which it was derived (Figure 5, left). A second determination of *Hsp70A* mRNA confirmed this outcome, after which the presence of the transposon at −97 was reverified by PCR and Southern hybridization.

Total Hsp70 protein levels approximately corresponded to these findings (Figure 6). In the 1IIa and US-4 strains, Hsp70 protein was similar in abundance after a 37° heat shock for 30 min. In two strains in which *Hsp70A* mRNA was reduced, Hsp70 protein was also reduced. By 30 min after heat shock, however, the difference in Hsp70 levels between these strains and US-4 had decreased. These strains exhibited similar levels of another *Hsp70* family member, Hsp68, after heat shock according to 2-D PAGE (data not shown).

Thermotolerance: As discussed above, genesis of the *Hsp70* transposon lines involved crossing two parental strains and mobilization of the original transposon. Both procedures and differing genetic backgrounds may affect thermotolerance, as is evident in four arbitrarily chosen pairs of strains derived from separate US-4 crosses. In all pairs, basal thermotolerance was greater than in US-4 (Figure 7). In each pair, one strain had lost the original *EPgy2* transposon (detectable by white eye color) and the other included an *EPgy2* transposon that had inserted outside the *Hsp70* genes and proximal flanking sequence. In each case, the transposon insertion strain had greater basal thermotolerance than its transposon-less counterpart. Likewise, all tested strains with insertions into *Hsp70A* had greater basal thermotolerance than their parental strain (Figure 7 and strains 111IIa, 30IIb, and 310II; data not shown). These differences are obviously not attributable to *Hsp70*, especially because Hsp70 is typically

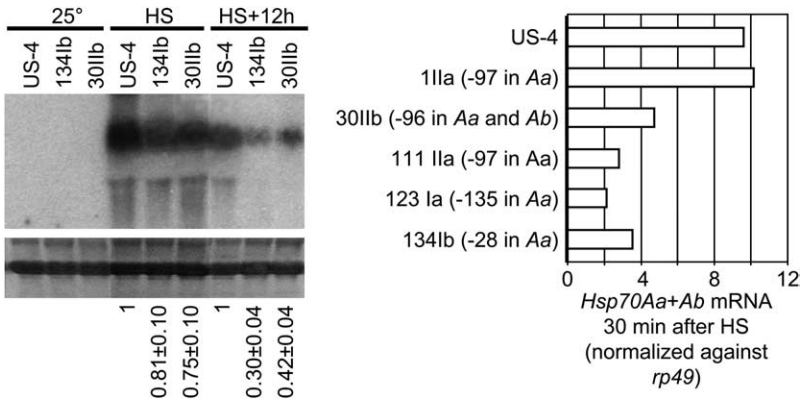

FIGURE 5.—Local transposition into *Hsp70A* promoters affects total *Hsp70* mRNA, but not consistently. (Left) Total mRNA from all six *Hsp70* genes. Heat shock was 37° for 30 min. Within each treatment, mRNA abundance in a strain with transposition into *Hsp70Aa* (134Ib) or into both *Hsp70A* genes (30IIb) was compared to that for controls (US-4 strain) via densitometry; mean ± standard error of relative abundance is indicated below the corresponding lane. (Right) Sum of *Hsp70Aa* and *Hsp70Ab* mRNA. The mRNA abundance in strains with one to two transpositions into *Hsp70A* was compared to that for controls (US-4 strain) via quantitative RT–PCR and normalized against *rp49* mRNA; means of three replicate determinations.

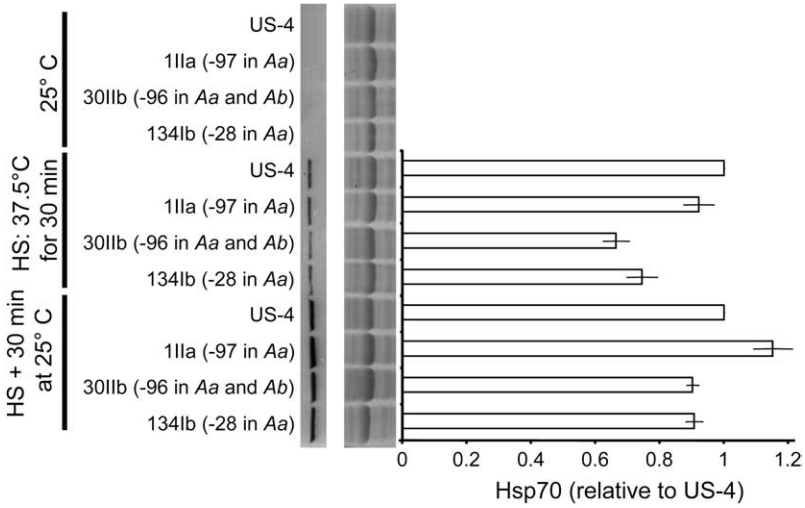

FIGURE 6.—Hsp70 protein in strains with transposition into *Hsp70A* and into a control strain (US-4). Images (rotated 90°) are a total Hsp70 immunoblot with antibody 7FB (left) and the corresponding Coomassie-stained membrane. (Right) Hsp70 abundance compared to that for controls (US-4 strain) via densitometry; mean ± standard error of relative abundance in four independent experiments is indicated.

undetectable before heat shock (VELAZQUEZ *et al.* 1983). By contrast, when US-4 and three *Hsp70* transposon strains derived from it underwent heat pretreatment before heat shock, the improvement in thermotolerance was greater in US-4 than in the *Hsp70* transposon-bearing strains. This difference was not evident for heat shocks of 40° or greater, at which survival was <20% (data not shown).

DISCUSSION

Transposition: Local transposition is an effective means of generating mutations in a target gene (TIMAKOV *et al.* 2002) and has been a key component of the Drosophila Gene Disruption Project (BELLEN *et al.* 2004). Here we show that the technique of TIMAKOV *et al.* (2002) can be used to define insertion site preference at the level of the individual nucleotide.

As has long been known, *P* elements do not insert at random in transposition experiments or screens, but preferentially into the 5′-flanking region of coding sequence (SPRADLING *et al.* 1995). Furthermore, in addition to this general preference are hotspots for *P* insertion, either into specific genes or regions of genes. The exact basis for this behavior is presently unknown, but "presumably" involves some combination of favorable chromatin accessibility, DNA target sequence, bound proteins, and relationship to the transposon's starting site (BELLEN *et al.* 2004). These features are consistent with several correlates of transposition frequency, including the transcriptional activity of the target gene and DNase hypersensitivity (VOELKER *et al.* 1990). The

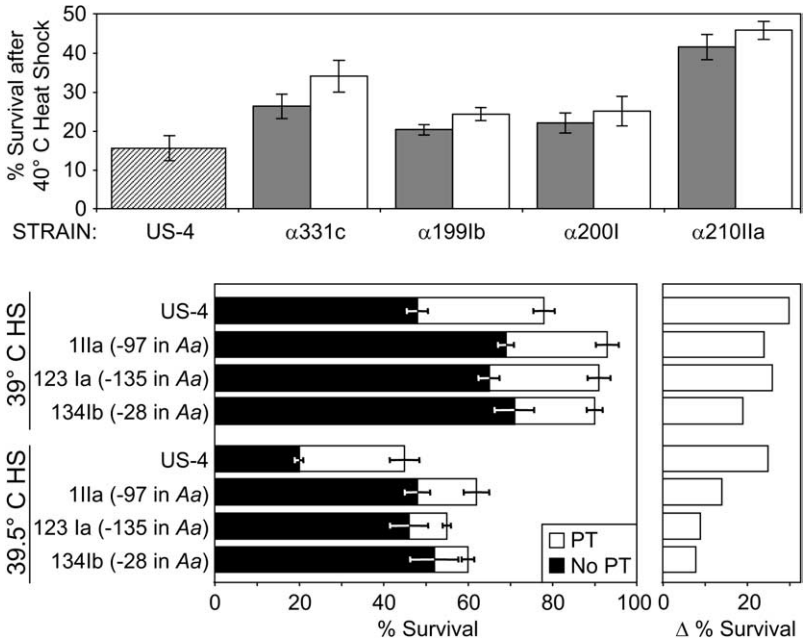

FIGURE 7.—Local transposition into the *Hsp70A* promoter decreases inducible but not basal thermotolerance. (Top) Effect on basal thermotolerance in four pairs of strains and in the strain from which these were derived (US-4). Each pair of lines was founded from segregants (recognizable by eye color) from an independent cross of US-4 with Delta2-3, one (shaded bar) carrying a novel insertion not in *Hsp70* and one (open bar) from which the *EPgy2* element had been lost. All pairs had greater thermotolerance than did US-4. (Bottom) Thermotolerance in strains with transposition into *Hsp70Aa* and in the strain from which they were derived (US-4). Plotted are means for flies with (open bars) or without (solid bars) pretreatment (35° for 30 min) before heat shock. The difference of means with and without thermotolerance is replotted at the right.

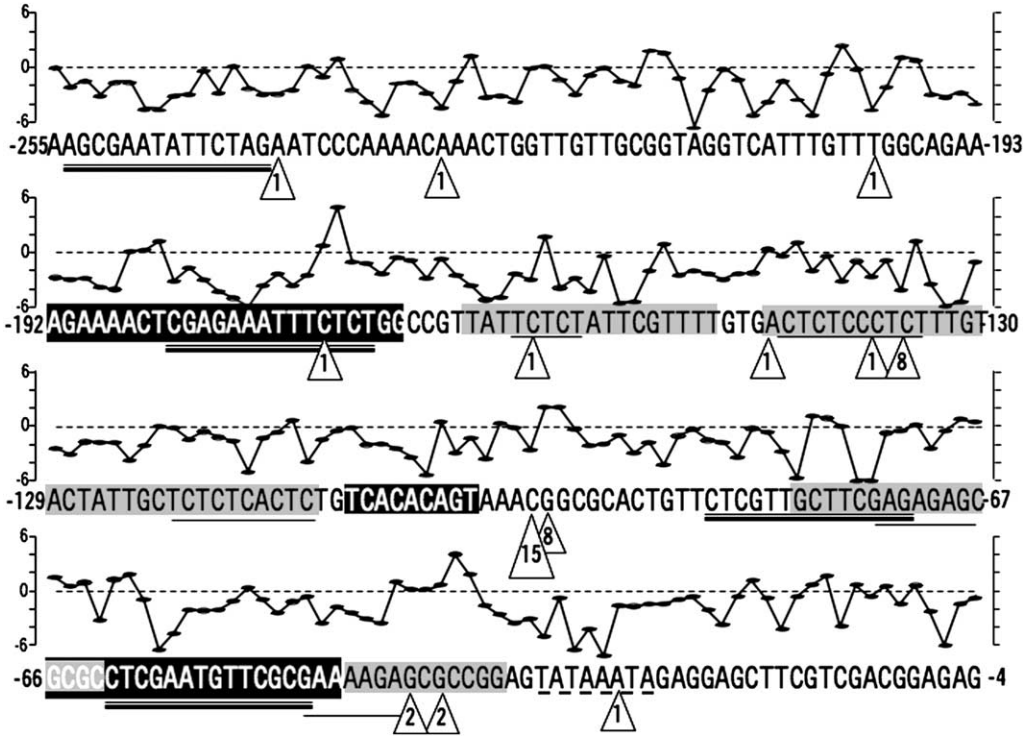

FIGURE 8.—Annotation of the *Hsp70* proximal promoter sequence, indicating locations of *EPgy2* element insertions from this study (triangles indicating the number of independent insertions from both starting sites), HSEs (double underlining), GAGA-factor-binding sites (single underlining), regions protected by GAF (shading) (GILMOUR *et al.* 1989), and DNase hypersensitive sites (black background with white text) (WEBER *et al.* 1997). Above each nucleotide the corresponding score of the 14-bp window extending 3 bases upstream and 10 bases downstream is indicated, calculated from a position weight matrix (SOSINSKY *et al.* 2003) derived from the training set of JULIAN (2003).

primary DNA sequence of the target site (as opposed to its accessibility and physical structure) seems a relatively unimportant determinant of transposition frequency (LIAO *et al.* 2000). In conclusion, the lack of exactitude in this summary should be evident.

Remarkably, more than half of the local transpositions into *Hsp70*—23 in total—are into one of the same two nucleotides (−96 and −97) in the *Hsp70Aa* and *Hsp70Ab* genes. Independent repeated transposition into specific nucleotides is not unprecedented (TOWER *et al.* 1993). The most spectacular example, however, is for CG9894, which received >700 independent hits in the Drosophila Gene Disruption Project, most of which were at specific nucleotides (BELLEN *et al.* 2004; Figure 4). As BELLEN *et al.* (2004, p. 778) state, "it may be possible to use insertion preferences as tools to probe chromosome organization and function." Indeed, the insertions into −96 and −97 in the *Hsp70Aa* and *Hsp70Ab* genes implicate several specific mechanisms.

First, the regions are in the 5′-flanking regions of heat-shock genes (SPRADLING *et al.* 1995). In this study, transposition into either the coding or 3′-flanking sequences of *Hsp70Aa* and *Hsp70Ab* never occurred, despite their sometimes-greater proximity to the starting transposon. This outcome contrasts to that for neighboring genes. As a class, heat-shock genes exhibit several distinctive features (see Introduction), of which many maintain these genes' proximal promoters in a decondensed, nucleosome-free architecture, which in principle should make the chromatin accessible to the transposition apparatus. Indeed, in the Drosophila Gene Disruption Project, "heat-shock genes" exceeded numerous other gene classes in their susceptibility to *P*-element insertion (BELLEN *et al.* 2004). In addition, *Hsp70* is expressed when germ cells develop (BOUTANAEV *et al.* 2002).

Second, the focal nucleotides, −96 and −97, are in a unique region of the *Hsp70* promoter (Figure 8). The attractiveness of the *Hsp* promoter as a model of environmental control of gene expression has yielded numerous detailed studies of this promoter's architecture. *In vivo*, much of the proximal *Hsp70* promoter is occupied by GAF, TATA box-binding factor, and their associated protein complexes (FARKAS *et al.* 2000; LEBEDEVA *et al.* 2005). A given GAF, in addition to participating in the chromatin-remodeling complex, can interact with other GAFs to organize chromatin into nucleosome-like domains in which the chromatin wraps around the GAFs (GEORGEL 2005). These structures prospectively present a distinctive chromatin conformation to transposase complexes. Nucleotides −96 and −97 are in the middle of a window (from ∼−80 to −111) flanked by GAF-binding sites but unprotected by bound GAF (GILMOUR *et al.* 1989; WEBER *et al.* 1997; GEORGEL 2005). Other such windows exist, but none are so large. Indeed, positions −109 to −100 correspond to DH2, the most hypersensitive of the DNase hypersensitive sites in the proximal promoter of Hsp70 (WU 1984; WEBER *et al.* 1997).

Finally, the sequence centered at −96 and −97 may favor transposition. O'HARE and RUBIN (1983) reported a consensus sequence of GGCCAGAC, but with considerable variation from the consensus tolerated. Physical and bioinformatic analyses have implicated GC

richness as contributing to insertion site preference, with six triplets (CAG, CTG, GAC, GCC, GGC, and GTC) and four dinucleotides (CC, GC, GG, and GT) often present. Fourteen base pairs (the 8-bp insertion site and 3 bp on either side) are a palindrome with a distinctive hydrogen bonding pattern (Liao *et al.* 2000). Recently Julian (2003) analyzed 795 *P* transpositions from the Drosophila Gene Disruption Project in detail and detected a preference for A/T at either end of the 14 bp. The insertion sites at −96 and −97 match Julian's consensus site at many positions, including the A/Ts in the specified positions. From Julian's (2003) training set, we have calculated a position weight matrix (Sosinsky *et al.* 2003) for this sequence and have scored a sliding window of 14 bp along the *Hsp70* promoter. Many (but not all) of the transposon insertion sites, including −96 or −97, are at or within 3 bp of local maxima for the score.

This analysis clearly cannot explain every local transposition into *Hsp70.* A site of eight independent insertions, −135, is in a region normally protected by GAF binding, not particularly hypersensitive, and dissimilar to Julian's (2003) consensus site.

Our prior discoveries of naturally occurring transpositions into Hsp70 genes are entirely consistent with these patterns. To date, we have reported on three *P* and one *Jockey* element in the proximal promoters of the *Hsp70B* genes and on a degenerate transposon ("56H8") and an *HMS Beagle* element in the region between the *Hsp70A* coding sequences (Michalak *et al.* 2001; Zatsepina *et al.* 2001; Bettencourt *et al.* 2002; Lerman *et al.* 2003; Lerman and Feder 2005). In this study, however, only *Hsp70A*, and not *Hsp70B*, was targeted by local transposition although the proximal promoters of *Hsp70* genes in these two clusters are identical for much of their length. We suggest that the latter cluster was simply out of range of local transposition.

Phenotypes of insertion lines: The *Hsp70* promoter is sensitive to mutations in the number and spacing of HSEs. Accordingly, we have suggested that transposition into the *Hsp70* promoter is a natural and normal source of evolutionary variation, which natural selection can fix or purge depending on the need for high levels of Hsp70 protein (Lerman *et al.* 2003). Tolerance of high temperatures is proportional to cellular Hsp70 levels, within limits (Krebs and Feder 1998). Hsp70 is a protein with numerous functions, however, many of which require stringent regulation of Hsp70 levels, and so high cellular concentrations of Hsp70 can be deleterious (Lerman *et al.* 2003). Depending on the frequency and severity of heat shock, high or low levels of Hsp70 may be beneficial to Drosophila (Lerman *et al.* 2003).

Consistent with this reasoning, natural populations of Drosophila harbor *Hsp70* alleles with *P* elements (or other transposons) in their proximal promoters (Michalak *et al.* 2001; Zatsepina *et al.* 2001; Bettencourt *et al.* 2002; Lerman *et al.* 2003; Lerman and Feder 2005). These segregate as alleles in nature and are at high frequencies, which Petrov *et al.* (2003) and others have viewed as indicative of positive selection. The frequency of transposon-bearing alleles, moreover, varies along ecological gradients in a manner consistent with selection (Michalak *et al.* 2001; Bettencourt *et al.* 2002). Other heat-shock genes also harbor numerous transposons in their proximal promoters (Franchini *et al.* 2004; our unpublished data).

Although *P* elements in particular may initially have large (and negative) effects and lead to ongoing mutagenesis upon first invasion of a Drosophila genome, selection readily leads to suppression of *P* transposition, degradation of the elements themselves, or co-evolution of the host genes enabling function despite (or contingent on) the transposons (Pinsker *et al.* 2001). We know only that the naturally occurring *P* elements invaded the *D. melanogaster* genome 50–200 years ago (Pinsker *et al.* 2001), and not how their phenotypes have evolved since their insertion into *Hsp70* promoters. The *de novo* insertions into the *Hsp70A* promoters provide an opportunity to recapitulate the original phenotypes of such elements. At the level of gene expression, the *EPgy2* elements almost always reduced the expression of the host gene, consistent with the requirement of the wild-type promoter sequence for full-strength expression of *Hsp70* genes (see above). The one exception, strain 1IIa, is enigmatic. An important nuance is that the *D. melanogaster* strains in this study have six nearly identical *Hsp70* copies. Insertional mutagenesis of one to two *Hsp70* copies might therefore have modest effects on total *Hsp70* mRNA. Indeed, in the mutant lines total *Hsp70* mRNA levels (Figure 4) and total Hsp70 protein levels (Figure 5) typically decreased 15–30% relative to controls after heat shock. Correspondingly, inducible thermotolerance was lower in controls (Figure 6). Previous work with natural and mutant lines has shown that such reductions are accompanied by increases in fitness components in the absence of stress (Lerman *et al.* 2003). This latter impact may account for the perpetuation of *P* elements in *Hsp* promoters in natural populations.

Finally, the *Hsp70* transposon strains and others (Gong and Golic 2004) may provide a useful test bed for emerging models of the regulation of *Hsp70* expression (Rieger *et al.* 2005). The six *Hsp70* genes each include four HSEs in their proximal promoter, and heat-shock protein levels are predicted to be highly sensitive to concentrations of activated HSF (Sarge *et al.* 1993; Rieger *et al.* 2005) and its occupancy of HSEs. As discussed above, transposons in hsp promoters may reduce gene expression by disrupting the native promoter conformation and binding HSF. Paradoxically, however, transposons in specific *Hsp70* genes may increase expression of nonaffected *Hsp70* genes by increasing the amount of available HSF. Because the 87A7 and the 87C1 *Hsp70* clusters show dramatically different kinetics and spatial patterns of expression

(Lakhotia and Prasanth 2002), such redistribution of HSF might be highly consequential. This suggestion obviously awaits future examination.

We thank Elena Zelentsova for performing *in situ* hybridization to confirm the localization of inserts, Ping Zhang for methodological advice, the Berkeley Drosophila Gene Disruption Project for producing the transposon source strains, Jean-Claude Walser for comments on the manuscript, and David S. Gilmour and Allan C. Spradling for detailed discussion of the *Hsp70* promoter and transposition, respectively. Spradling suggested that disruption of one HSE might redistribute HSF to other HSEs. The work was supported by a Russian Academy grant for Molecular and Cellular Biology to M.B.E., Russian Grants for Basic Science 06-04-48993-a and 06-04-48854-a, and National Science Foundation grant IBN03-16627. Bing Chen was supported by the China Scholarship Council.

LITERATURE CITED

Amin, J., M. Fernandez, J. Ananthan, J. T. Lis and R. Voellmy, 1994 Cooperative binding of heat shock transcription factor to the *Hsp70* promoter in vivo and in vitro. J. Biol. Chem. **269:** 4804–4811.

Aminetzach, Y. T., J. M. Macpherson and D. A. Petrov, 2005 Pesticide resistance via transposition-mediated adaptive gene truncation in Drosophila. Science **309:** 764–767.

Bailey, J. A., G. Liu and E. E. Eichler, 2003 An Alu transposition model for the origin and expansion of human segmental duplications. Am. J. Hum. Genet. **73:** 823–834.

Becker, J., and E. A. Craig, 1994 Heat-shock proteins as molecular chaperones. Eur. J. Biochem. **219:** 11–23.

Bellen, H. J., R. W. Levis, G. C. Liao, Y. C. He, J. W. Carlson *et al.*, 2004 The BDGP gene disruption project: single transposon insertions associated with 40% of Drosophila genes. Genetics **167:** 761–781.

Bettencourt, B. R., and M. E. Feder, 2002 Rapid concerted evolution via gene conversion at the Drosophila *Hsp70* genes. J. Mol. Evol. **54:** 569–586.

Bettencourt, B. R., I. Kim, A. A. Hoffmann and M. E. Feder, 2002 Response to natural and laboratory selection at the Drosophila *Hsp70* genes. Evolution **56:** 1796–1801.

Bogwitz, M. R., H. Chung, L. Magoc, S. Rigby, W. Wong *et al.*, 2005 Cyp12a4 confers lufenuron resistance in a natural population of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **102:** 12807–12812.

Boutanaev, A. M., A. I. Kalmykova, Y. Y. Shevelyou and D. I. Nurminsky, 2002 Large clusters of co-expressed genes in the Drosophila genome. Nature **420:** 666–669.

Daborn, P. J., J. L. Yen, M. R. Bogwitz, G. Le Goff, E. Feil *et al.*, 2002 A single P450 allele associated with insecticide resistance in Drosophila. Science **297:** 2253–2256.

Evgen'ev, M. B., O. G. Zatsepina, D. Garbuz, D. N. Lerman, V. Veleikodvorskaja *et al.*, 2004 Evolution and arrangement of the *hsp70* gene cluster in two closely-related species of the *virilis* group of Drosophila. Chromosoma **113:** 223–232.

Farkas, G., B. A. Leibovitch and S. C. Elgin, 2000 Chromatin organization and transcriptional control of gene expression in Drosophila. Gene **253:** 117–136.

Franchini, L. F., E. W. Ganko and J. F. McDonald, 2004 Retrotransposon-gene associations are widespread among *D. melanogaster* populations. Mol. Biol. Evol. **21:** 1323–1331.

Garbuz, D. G., V. B. Molodtsov, V. V. Velikodvorskaia, M. B. Evgen'ev and O. G. Zatsepina, 2002 Evolution of the response to heat shock in genus *Drosophila*. Russ. J. Genet. **38:** 925–936.

Garbuz, D., M. B. Evgenev, M. E. Feder and O. G. Zatsepina, 2003 Evolution of thermotolerance and the heat-shock response: evidence from inter/intraspecific comparison and interspecific hybridization in the *virilis* species group of Drosophila. I. Thermal phenotype. J. Exp. Biol. **206:** 2399–2408.

Georgel, P. T., 2005 Chromatin potentiation of the *Hsp70* promoter is linked to GAGA-factor recruitment. Biochem. Cell Biol. **83:** 555–565.

Gilmour, D. S., G. H. Thomas and S. C. Elgin, 1989 Drosophila nuclear proteins bind to regions of alternating C and T residues in gene promoters. Science **245:** 1487–1490.

Golic, K. G., 1994 Local transposition of *P* elements in *Drosophila melanogaster* and recombination between duplicated elements using a site-specific recombinase. Genetics **137:** 551–563.

Gong, W. J., and K. G. Golic, 2004 Genomic deletions of the *Drosphila melanogaster Hsp70* genes. Genetics **168:** 1467–1476.

Jeanmougin, F., J. D. Thompson, M. Gouy, D. G. Higgins and T. J. Gibson, 1998 Multiple sequence alignment with Clustal X. Trends Biochem. Sci. **23:** 403–405.

Julian, A. M., 2003 Use of bioinformatics to investigate and analyze transposable element insertions in the genomes of *Caenorhabditis elegans* and *Drosophila melanogaster*, and into the target plasmid *pGDV1*. Master's Thesis, Texas A&M University, College Station, TX.

Kapitonov, V. V., and J. Jurka, 2003 Molecular paleontology of transposable elements in the *Drosophila melanogaster* genome. Proc. Natl. Acad. Sci. USA **100:** 6569–6574.

Karpov, V. L., O. V. Preobrazhenskaya and A. D. Mirzabekov, 1984 Chromatin structure of *Hsp70* genes, activated by heat shock: selective removal of histones from the coding region and their absence from the 5′ region. Cell **36:** 423–431.

Kazazian, H. H., 2004 Mobile elements: drivers of genome evolution. Science **303:** 1626–1632.

Krebs, R. A., and M. E. Feder, 1998 Hsp70 and larval thermotolerance in *Drosophila melanogaster:* How much is enough and when is more too much? J. Insect Physiol. **44:** 1091–1101.

Lakhotia, S. C., and K. V. Prasanth, 2002 Tissue- and development-specific induction and turnover of *Hsp70* transcripts from loci 87A and 87C after heat shock and during recovery in *Drosophila melanogaster*. J. Exp. Biol. **205:** 345–358.

Lebedeva, L. A., E. N. Nabirochkina, M. M. Kurshakova, F. Robert, A. N. Krasnov *et al.*, 2005 Occupancy of the Drosophila *Hsp70* promoter by a subset of basal transcription factors diminishes upon transcriptional activation. Proc. Natl. Acad. Sci. USA **102:** 18087–18092.

Lerman, D. N., and M. E. Feder, 2005 Naturally occurring transposable elements disrupt *Hsp70* promoter function in *Drosophila melanogaster*. Mol. Biol. Evol. **22:** 776–783.

Lerman, D. N., P. Michalak, A. B. Helin, B. R. Bettencourt and M. E. Feder, 2003 Modification of heat-shock gene expression in *Drosophila melanogaster* populations via transposable elements. Mol. Biol. Evol. **20:** 135–144.

Lewis, A. P., and J. F. Y. Brookfield, 1987 Movement of *Drosophila melanogaster* transposable elements other than P elements in a P-M hybrid dysgenic cross. Mol. Gen. Genet. **208:** 506–510.

Li, B., J. A. Weber, Y. Chen, A. L. Greenleaf and D. S. Gilmour, 1996 Analyses of promoter-proximal pausing by RNA polymerase II on the *Hsp70* heat shock gene promoter in a Drosophila nuclear extract. Mol. Cell. Biol. **16:** 5433–5443.

Liao, G. C., E. J. Rehm and G. M. Rubin, 2000 Insertion site preferences of the P transposable element in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **97:** 3347–3351.

Lis, J., and C. Wu, 1993 Protein traffic on the heat shock promoter: parking, stalling, and trucking along. Cell **74:** 1–4.

Marsano, R. M., R. Caizzi, R. Moschetti and N. Junakovic, 2005 Evidence for a functional interaction between the Bari1 transposable element and the cytochrome P450 cyp12a4 gene in *Drosophila melanogaster*. Gene **357:** 122–128.

Mason, P. B., and J. T. Lis, 1997 Cooperative and competitive protein interactions at the *Hsp70* promoter. J. Biol. Chem. **272:** 33227–33233.

McCollum, A., E. Ganko, P. Barrass, J. Rodriguez and J. McDonald, 2002 Evidence for the adaptive significance of an LTR retrotransposon sequence in a *Drosophila* heterochromatic gene. BMC Evol. Biol. **19:** 5.

McGarry, T. J., and S. Lindquist, 1985 The preferential translation of Drosophila *Hsp70* mRNA requires sequences in the untranslated leader. Cell **42:** 903–911.

Michalak, P., I. Minkov, A. Helin, D. N. Lerman, B. R. Bettencourt *et al.*, 2001 Genetic evidence for adaptation-driven incipient speciation of *Drosophila melanogaster* along a microclimatic contrast in "Evolution Canyon," Israel. Proc. Natl. Acad. Sci. USA **98:** 13195–13200.

Nacheva, G. A., D. Y. Guschin, O. V. Preobrazhenskaya, V. L. Karpov, K. K. Ebralidse *et al.*, 1989 Change in the pattern of histone binding to DNA upon transcriptional activation. Cell **58:** 27–36.

O'Brien, T., and J. T. Lis, 1991 RNA polymerase II pauses at the 5′ end of the transcriptionally induced Drosophila *Hsp70* gene. Mol. Cell. Biol. **11:** 5285–5290.

O'Brien, T., R. C. Wilkins, C. Giardina and J. T. Lis, 1995 Distribution of GAGA protein on Drosophila genes in vivo. Genes Dev. **9:** 1098–1110.

O'Hare, K., and G. M. Rubin, 1983 Structures of *P* transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34:** 25–35.

Petrov, D. A., Y. T. Aminetzach, J. C. Davis, D. Bensasson and A. E. Hirsh, 2003 Size matters: non-LTR retrotransposable elements and ectopic recombination in Drosophila. Mol. Biol. Evol. **20:** 880–892.

Pinsker, W., E. Haring, S. Hagemann and W. J. Miller, 2001 The evolutionary life history of *P* transposons: from horizontal invaders to domesticated neogenes. Chromosoma **110:** 148–158.

Puig, M., M. Caceres and A. Ruiz, 2004 Silencing of a gene adjacent to the breakpoint of a widespread Drosophila inversion by a transposon-induced antisense RNA. Proc. Natl. Acad. Sci. USA **101:** 9013–9018.

Rieger, T. R., R. I. Morimoto and V. Hatzimanikatis, 2005 Mathematical modeling of the eukaryotic heat-shock response: dynamics of the *Hsp70* promoter. Biophys. J. **88:** 1646–1658.

Sarge, K. D., S. P. Murphy and R. I. Morimoto, 1993 Activation of heat shock gene transcription by heat shock factor 1 involves oligomerization, acquisition of DNA-binding activity, and nuclear localization and can occur in the absence of stress. Mol. Cell. Biol. **13:** 1392–1407.

Schlenke, T. A., and D. J. Begun, 2004 Strong selective sweep associated with a transposon insertion in *Drosophila simulans*. Proc. Natl. Acad. Sci. USA **101:** 1626–1631.

Sosinsky, A., C. P. Bonin, R. S. Mann and B. Honig, 2003 Target Explorer: an automated tool for the identification of new target genes for a specified set of transcription factors. Nucleic Acids Res. **31:** 3589–3592.

Spradling, A. C., D. M. Stern, I. Kiss, J. Roote, T. Laverty *et al.*, 1995 Gene disruptions using P transposable elements: an integral component of the Drosophila Genome Project. Proc. Natl. Acad. Sci. USA **92:** 10824–10830.

Timakov, B., X. Liu, I. Turgut and P. Zhang, 2002 Timing and targeting of *P*-element local transposition in the male germline cells of *Drosophila melanogaster*. Genetics **160:** 1011–1022.

Tower, J., G. H. Karpen, N. Craig and A. C. Spradling, 1993 Preferential transposition of Drosophila *P* elements to nearby chromosomal sites. Genetics **133:** 347–359.

Tsukiyama, T., P. B. Becker and C. Wu, 1994 ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. Nature **367:** 525–532.

Udvardy, A., E. Maine and P. Schedl, 1985 The 87A7 chromomere. Identification of novel chromatin structures flanking the heat shock locus that may define the boundaries of higher order domains. J. Mol. Biol. **185:** 341–358.

Velazquez, J. M., S. Sonoda, G. Bugaisky and S. Lindquist, 1983 Is the major Drosophila heat shock protein present in cells that have not been heat shocked? J. Cell Biol. **96:** 286–290.

Voelker, R. A., J. Graves, W. Gibson and M. Eisenberg, 1990 Mobile element insertions causing mutations in the Drosophila *suppressor of sable* locus occur in DNase I hypersensitive subregions of 5′-transcribed nontranslated sequences. Genetics **126:** 1071–1082.

Weber, J. A., D. J. Taxman, Q. Lu and D. S. Gilmour, 1997 Molecular architecture of the *Hsp70* promoter after deletion of the TATA box or the upstream regulation region. Mol. Cell. Biol. **17:** 3799–3808.

Wu, C., 1984 Two protein-binding sites in chromatin implicated in the activation of heat-shock genes. Nature **309:** 229–234.

Xing, H. Y., D. C. Wilkerson, C. N. Mayhew, E. J. Lubert, H. S. Skaggs *et al.*, 2005 Mechanism of *Hsp70i* gene bookmarking. Science **307:** 421–423.

Zatsepina, O. G., V. V. Velikodvorskaia, V. B. Molodtsov, D. Garbuz, D. N. Lerman *et al.*, 2001 A *Drosophila melanogaster* strain from sub-equatorial Africa has exceptional thermotolerance but decreased Hsp70 expression. J. Exp. Biol. **204:** 1869–1881.

Communicating editor: K. G. Golic