MOLECULAR AND CELLULAR BIOLOGY, Jan. 1989, p. 332–335
0270-7306/89/010332-04$02.00/0

Vol. 9, No. 1

Perturbation of Chromatin Architecture on Ecdysterone Induction of *Drosophila melanogaster* Small Heat Shock Protein Genes

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Received 17 June 1988/Accepted 21 October 1988

Alterations in the pattern of DNase I hypersensitivity were observed on ecdysterone-stimulated transcription of *Drosophila melanogaster* small heat shock protein genes. Perturbations were induced near *hsp27* and *hsp22*, coupled with an extensive domain of chromatin unfolding in the intergenic region between *hsp23* and the developmentally regulated gene 1. These regions represent candidates for ecdysterone regulatory interactions.

Locus 67B1 of the *Drosophila melanogaster* genome contains seven genes: four that are strongly heat induced (*hsp22*, *hsp23*, *hsp26*, and *hsp27*) and three others (genes 1, 2, and 3) which respond only weakly to hyperthermic shock (1). In addition to being heat inducible, all these genes have been found to be developmentally regulated in a complex manner throughout the life cycle of the fly. In late third instar larvae and prepupae, all four of the small heat shock protein genes and both genes 1 and 3 are expressed (15, 18, 23) at a time coinciding with elevated titers of the insect steroid molting hormone ecdysterone. Ecdysterone directly stimulates the synthesis of the small heat shock protein gene mRNAs and proteins in both the Schneider 3 (S3) *D. melanogaster* cell line and isolated imaginal discs (12, 13). In addition, *hsp26*, *hsp27*, and gene 2 are expressed in the gonads of adult flies (10, 19, 28), but preliminary evidence implicates mechanisms for regulation at the larval and prepupal stages that are distinct from those for testicular (10, 19) or ovarian (5, 6) adult expression. Thus, regulation of the genes at this locus involves a complex set of controls acting to allow heat-induced, developmental, and gonad-specific expression.

Studies of the patterns of chromatin DNase I hypersensitivity around genes both before and following induction (or in cell types in which the gene either is or is not expressed) have shown that perturbations most often occur at or very close to gene regulatory sequences (4, 11). Investigation of such perturbation occurring at the small heat shock protein genes on thermal induction revealed the establishment of "footprints" (3) over those sequences previously identified by expression analysis as important for heat shock induction (20, 27). Here we have pursued such a chromatin analysis with cells that express these genes in response to ecdysterone as a means of identifying potentially important regions of the locus mediating the developmental response to hormone.

S3 cells were grown in Schneider medium plus 12.5% fetal bovine serum at 25°C and were exposed to 2.5 μM ecdysterone for 24 h. Non-hormonally induced control cells were treated identically. Nuclei were isolated essentially as previously described (2, 3, 26) and were immediately subjected to a graded series of DNase I digestions at 25°C for 3 min (26). DNA was purified by standard techniques, and the locations of DNase I hypersensitive (DH) sites were determined by the indirect end-labeling procedure (17, 25). Restriction enzyme-digested DNA was size fractionated by electrophoresis on 45-cm agarose gels at 4°C. DNA fragments were transferred to nitrocellulose, hybridized with randomly primed labeled probes (7, 8), stringently washed by methods described previously (3), and exposed to X-ray film.

The resulting autoradiograms allowed the high-resolution mapping of DH sites both before (control lanes; see Fig. 1 to

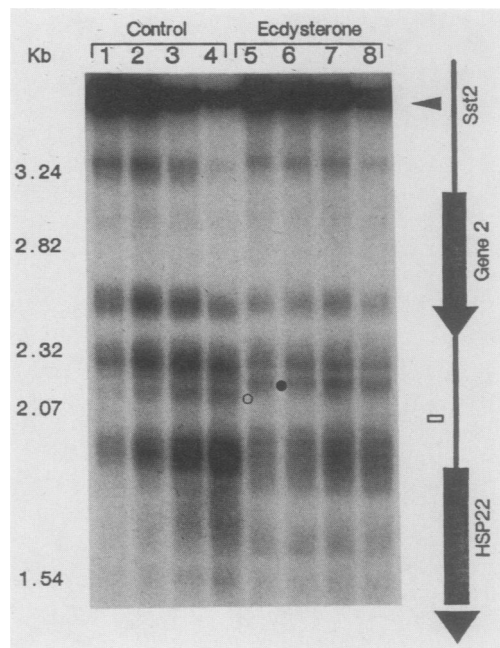

FIG. 1. DNase I hypersensitivity pattern upstream of *hsp22*. Samples were 5 μg of S3 DNA digested to completion with *Sst*II, resolved on a 1.5% agarose gel, and probed with the 859-bp *Sst*II-*Bgl*II fragment (see Fig. 4). Lanes 1 to 4 show graded DNase I digestions at 2.7, 5.7, 11, and 17 U/ml, respectively, of nuclei from control cells; and lanes 5 to 8 show graded DNase I digestions at 6.7, 8.0, 13, and 17 U/ml, respectively, of nuclei from ecdysterone-treated cells. Closed circle represents region of increased DNase I sensitivity (novel DH site); open circle represents the region which became less sensitive to DNase I on ecdysterone induction (footprint). The line drawing shows the transcriptional start site of *hsp22*, the body and direction of transcription (arrow) of the male-specific transcript of gene 2, and the distal *Sst*II site. The open box indicates the location of the proposed ecdysterone regulatory element for *hsp22* (14). Kb, Kilobases.

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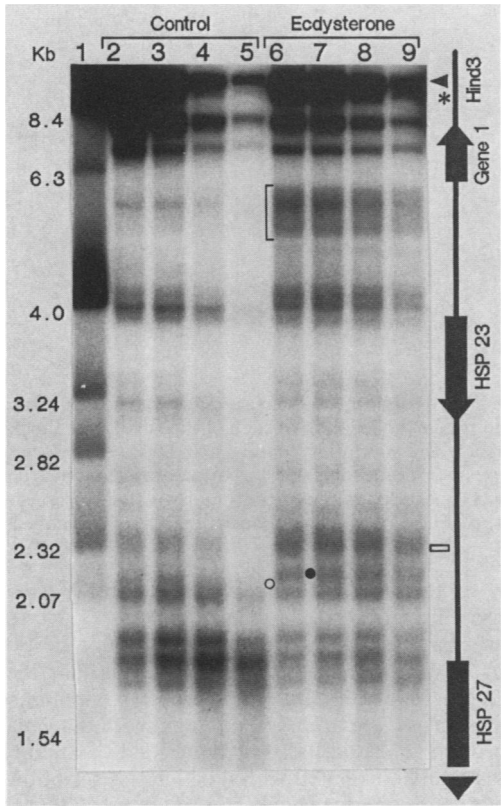

FIG. 2. DNase I hypersensitivity pattern upstream of *hsp27*. Samples were 5 μg of S3 DNA digested to completion with *Hin*dIII, resolved on a 1.4% agarose gel, and probed with the 670-bp *Hin*dIII-*Ava*I fragment (see Fig. 4). Lanes 2 to 5 show graded DNase I digestions of nuclei from control cells, and lanes 6 to 9 show graded DNase I digestions of nuclei from ecdysterone-treated cells, as in Fig. 1. Closed and open circles are as explained in the legend to Fig. 1, and the bracket indicates the region of major chromatin perturbation in the *hsp23*-gene 1 intergenic region. Lane 1 shows size markers of restriction digests of pBR322 and cloned portions of this locus used to determine relative locations of DH sites. The line drawing indicates the transcriptional start site of *hsp27*, the bodies and directions of transcription (arrows) of *hsp23* and gene 1, and the distal *Hin*dIII site; the asterisk indicates the location of distal *hsp26* DH regions. The open box indicates the location of the proposed ecdysterone regulatory dyad for *hsp27* (21). Kb, Kilobases.

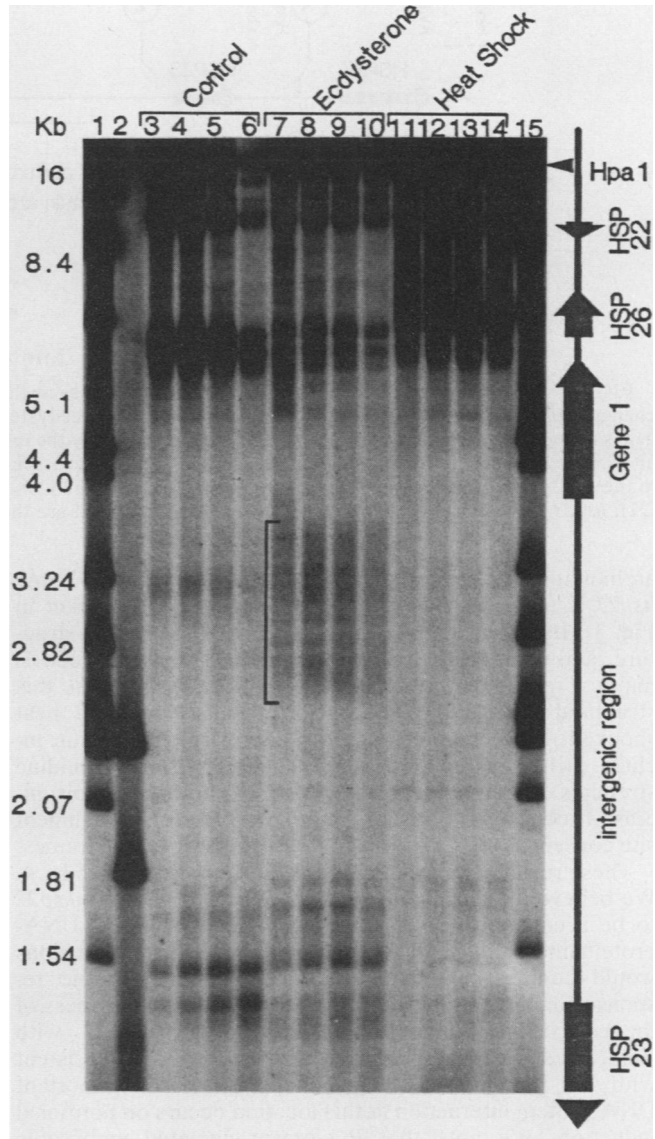

FIG. 3. DNase I hypersensitivity pattern of the *hsp23*-gene 1 intergenic region. Samples were 5 μg of S3 DNA digested to completion with *Hpa*I, resolved on a 1.2% agarose gel, and probed with the 394-bp *Hpa*I-*Xba*I fragment (see Fig. 4). Lanes 3 to 6 show graded DNase I digestions of control nuclei, and lanes 7 to 10 show graded DNase I digestions of nuclei from ecdysterone-treated cells, as in Fig. 1. Lanes 12 to 14 show graded DNase I digestions of nuclei from cells heat shocked for 45 min at 37°C. The bracket indicates the region of major chromatin architectural perturbation discussed in the text. Lanes 1, 2, and 15 show size markers of restriction digests of pBR322 and cloned portions of this locus. The line drawing shows the transcriptional start site of *hsp23* and the bodies and directions of transcription (arrows) of gene 1, *hsp26*, and *hsp22*, as well as the distal *Hpa*I site. Kb, Kilobases.

3) and after (ecdysterone lanes) induction of these genes by hormone. Previous studies showed that none of the DH sites to be discussed was a consequence of DNase I cutting preferences on protein-free DNA (3). Figure 1 reveals that the *hsp22* region underwent a specific change in chromatin architecture in response to ecdysterone. A distinct upstream region of DNase I insensitivity (footprint) was induced (open circle), completely ablating a DH site present in control cells at approximately −250 ± 30 base pairs (bp). This footprint is now flanked upstream by a novel DH site (closed circle) present only in ecdysterone-treated cells. Figure 2 reveals quantitative changes in hypersensitive regions upstream of *hsp27*. We note in particular that one of a series of upstream DH sites present in control cells became relatively insensitive to DNase I with exposure to hormone (denoted by the open circle in Fig. 2 and mapping to −400 ± 30 bp). Concurrently, a region immediately upstream of this became rather more DNase I sensitive (closed circle in Fig. 2), as did the preexisting DH site at about −550 bp (open box), when compared with control cells. Farther upstream of *hsp27*, visible alterations in the intergenic region between *hsp23* and gene 1 can be seen; these are discussed in detail below.

Although some qualitative changes are apparent in Fig. 3 in the vicinity of the *hsp23* cap site with ecdysterone treatment, there are no very noticeable changes in relative levels of DNase I sensitivity of the kind observed at *hsp22* and *hsp27*. However, a large perturbation in the chromatin

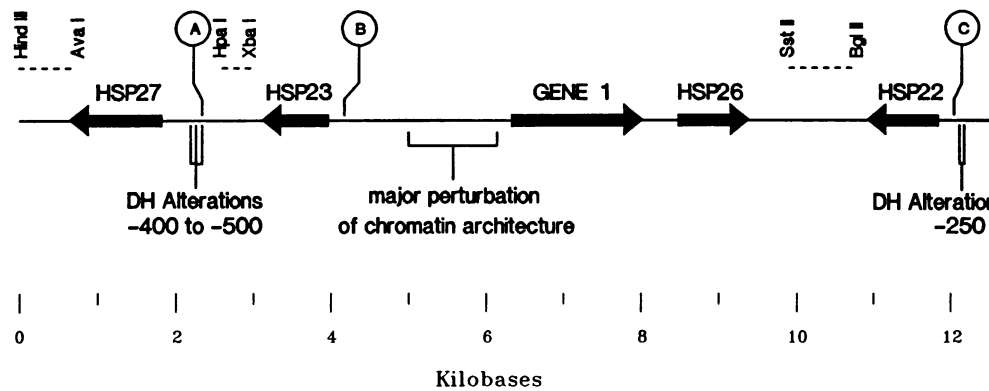

FIG. 4. Ecdysterone-induced chromatin structural changes at the small heat shock protein gene locus; a diagrammatic summary of the changes observed in *D. melanogaster* S3 cells in response to ecdysterone. The map shows the location of each of the small heat shock protein genes and gene 1; the direction of transcription is indicated by the relevant arrowhead. The alterations found 400 to 500 bp upstream of *hsp27* and 250 bp upstream of *hsp22* are both indicated, and the large bracket shows the position of the major perturbation of chromatin architecture in the *hsp23*-gene 1 intergenic region. The encircled A, B, and C indicate the positions of proposed ecdysterone regulatory regions at *hsp27* (21), *hsp23* (16), and *hsp22* (14), respectively. Also indicated are the probes (dashed lines) used in the indirect end-labeling analysis.

architecture of the intergenic region between gene 1 and *hsp23* is unmistakably present (depicted by the bracket in Fig. 3). Its extent (approximately 1.4 kilobases), fine structure, and location (beginning immediately distal to gene 1) make it particularly unusual. It is noteworthy that this structural change did not occur in cells that were heat shocked. Sequences of potential interest in this region include (i) several long alternating purine-pyrimidine stretches, (ii) numerous close matches to both the topoisomerase II consensus (22) and the bipartite scaffold attachment site consensus (9), and (iii) a number of open reading frames.

These results are diagrammatically summarized in Fig. 4. We believe the chromatin perturbations at *hsp22* and *hsp27* to be a consequence of ecdysterone-induced novel DNA-protein interactions, and thus we anticipate that both regions would contain candidate sequences for ecdysterone response elements involved in the hormone responsiveness of the genes. The elimination of a DH site at *hsp22*, with simultaneous appearance of an upstream site, is consistent with the notion that the establishment of an important DNA-protein interaction at this location occurs on hormonal induction. We note that P element-mediated embryonic transformation experiments have implicated a region within 70 bp of this footprinted region as important for *hsp22* developmental regulation (14). Virtually coincident with this chromatin perturbation is a consensus DNA element related to one deemed important on the basis of transient expression tests of *hsp23* regulation (16). Paradoxically, we saw little obvious chromatin perturbation of the region upstream of *hsp23* that contains this sequence, even though we used the same cell line in which the transient assays were performed. No perturbations were observed in the vicinity of *hsp26* (data not shown), even though it is known to be transcriptionally induced by ecdysterone in these cells (13). We interpret the changes observed at *hsp27* to be the probable result of newly established DNA-protein interactions occurring at either (or both) of the regions -400 ± 30 and -500 ± 30 bp, since both regions appear to be quite insensitive to DNase I and are flanked both distally and proximally by DH sites. The latter region encompasses sequences able to confer ecdysterone responsiveness on a heterologous gene in tissue culture cells (21). However, there are no sequences in this region of chromatin perturbation that match the *hsp23* consensus (16), nor are there good candidates for consensus matches to this *hsp27* element near the other small heat shock protein genes.

We are particularly intrigued by the major perturbation seen upstream of gene 1. One possibility is that there is a novel gene here. The extent of perturbation is reminiscent of that seen across the transcribed region of the small heat shock protein genes on thermal induction (3). Such changes are most readily correlated with the high transcription rate of these four genes on heat shock; it is noteworthy that they did not show this type of perturbation on hormonal induction (this work), a situation in which the rate of transcription is markedly lower (24). If a novel gene is present at this location, we would expect its rate of transcription to be extremely high on the basis of the chromatin phenotype. Preliminary tests for transcription within this region (unpublished results) have suggested a very low level of transcription on both hormonal and thermal induction but none in control cells. Further investigation of this is under way, but it should be noted that the chromatin perturbation was absent in heat-shocked cells (Fig. 3, lanes 11 to 14). A second possibility (not necessarily incompatible with or exclusive of the first) is that this region contains some form of ecdysterone-specific regulatory element, perhaps a stage- or tissue-specific enhancer, that might coordinate the expression of these genes through interaction with their individually distinctive ecdysterone response elements. Its location beween four divergent transcription units is intriguing (Fig. 4), the more so since we have detected gene 1 expression in S3 cells in response to ecdysterone (data not shown). Other possibilities for function can be envisaged, and our efforts are currently focused on delineating the basis for this unusual chromatin perturbation.

We thank Richard Southgate for kindly providing an unpublished sequence.

This work was supported by Public Health Service grant GM-37213 to I.L.C. from the National Institutes of Health and a graduate fellowship from the Albert J. Ryan Foundation to S.E.K.

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