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Rapid Changes in *Drosophila* Transcription after an Instantaneous Heat Shock

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Heat shock rapidly activates expression of some genes and represses others. The kinetics of changes in RNA polymerase distribution on heat shock-modulated genes provides a framework for evaluating the mechanisms of activation and repression of transcription. Here, using two methods, we examined the changes in RNA polymerase II association on a set of *Drosophila* genes at 30-s intervals following an instantaneous heat shock. In the first method, *Drosophila* Schneider line 2 cells were quickly frozen to halt transcription, and polymerase distribution was analyzed by a nuclear run-on assay. RNA polymerase transcription at the 5′ end of the *hsp70* gene could be detected within 30 to 60 s of induction, and by 120 s the first wave of polymerase could already be detected near the 3′ end of the gene. A similar rapid induction was found for the small heat shock genes (*hsp22*, *hsp23*, *hsp26*, and *hsp27*). In contrast to this rapid activation, transcription of the histone *H1* gene was found to be rapidly repressed, with transcription reduced by ~90% within 300 s of heat shock. Similar results were obtained by an in vivo UV cross-linking assay. In this second method, cell samples removed at 30-s intervals were irradiated with 40-μs bursts of UV light from a Xenon flash lamp, and the distribution of polymerase was examined by precipitating UV cross-linked protein-DNA complexes with an antibody to RNA polymerase II. Both approaches also showed the in vivo rate of movement of the first wave of RNA polymerase through the *hsp70* gene to be ~1.2 kb/min.

Transcriptional activation of *Drosophila* heat shock genes can occur within minutes of exposure to a heat shock stimulus. It was previously found that within 2 min of exposing *Drosophila* larvae to a temperature of 37°C a distinct puff could be seen at cytological position 63B (which contains the heat shock gene *hsp83*) and that this puff reached its maximum size within 5 min (1). Incorporation of [^{3}H]uridine was also found at the 87A and 87C loci (which together contain five copies of the *hsp70* gene) within 1 min of heat shock (1), suggesting a rapid transcriptional response. In support of this finding, mRNA transcribed from the *hsp70* gene can be detected as early as 4 min after shifting Schneider line 2 (SL2) cells to the full heat shock temperature, and an increase in the level of HSP70 can be detected as early as 8 to 12 min after heat shock (19).

Transcriptional induction of heat shock genes is mediated by heat shock factor (HSF). Each *Drosophila* heat shock gene contains a repeating array of a 5-bp unit (heat shock element [HSE]) which is the core binding sequence for HSF (for a review, see reference 20). In uninduced cells HSF is maintained in an inactive state and is unable to bind efficiently to HSEs in vitro (38). Heat shock induces multimerization of HSF, which coincides with its acquiring the ability to bind specifically to HSEs (38). HSF isolated from cells as early as 30 s after exposure to a heat shock is capable of binding to the upstream HSEs of *hsp83* in vitro (43). This indicates that the activation of transcription by HSF could potentially occur within seconds.

We were interested in examining how rapidly *Drosophila* heat shock gene transcription can occur after a rapid increase in temperature. The uninduced *Drosophila hsp70* gene contains an RNA polymerase II molecule that has initiated transcription but has paused after synthesizing 17 to 46 bases (9, 26, 28). This polymerase presumably resumes elongation into the body of the gene once the promoter becomes activated. Pausing of polymerase has also been detected at the 5′ ends of the *hsp26* (9, 26, 29), *hsp23* (25), and *hsp27* genes (26). A promoter that already contains an initiated polymerase could be capable of responding very rapidly to an increase in temperature. To measure the rapidity of this response, SL2 cells were exposed to an instantaneous heat shock (by adding an equal volume of 56°C tissue culture medium), samples were removed at 30-s intervals, and then they were rapidly frozen in liquid nitrogen. After isolation of the nuclei, polymerase distribution was examined at high resolution on a series of genes by a short in vitro nuclear run-on assay. The genes examined were heat-inducible genes (*hsp70*, *hsp22*, *hsp23*, *hsp26*, and *hsp27*), genes whose transcription is unaffected by heat shock (the core histone genes [7, 10]), and a gene whose transcription is repressed by heat shock (histone *H1* [7]). We also examined RNA polymerase II distribution by an in vivo UV cross-linking technique, in which covalently cross-linked protein-DNA complexes are immunoprecipitated with an antibody directed against RNA polymerase.

MATERIALS AND METHODS

Plasmids. Plasmids containing the core histone genes were as described earlier (24). Each of these plasmids was digested so that an entire core histone transcription unit would be on a single fragment or so that the transcription region was divided into two fragments, one containing a 5′ and the other a 3′ coding region.

The plasmid containing the *H1* gene is pUH1.9, and it was constructed by cloning a *Bgl*II-*Hpa*I fragment containing this coding region from cDm500 (18) into pUC19 digested with *Bam*HI and *Hin*cII (40).

The plasmid containing the *hsp70* gene is p70X2.6 (28).

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The plasmid containing the *hsp22* gene is pG3.22s, and it was constructed by cloning an ~1.05-kb *Xba*I-*Bam*HI fragment containing the *hsp22* gene from λbDm202 (5) into pGEM3 digested with *Xba*I-*Bam*HI (30).

The plasmid containing the *hsp23* gene is gP23ΔXs. This was constructed from pG3.23s (generated by cloning a *Pst*I fragment containing the *hsp23* transcription unit from λbDm202 [5] into *Pst*I-digested pGEM3 [30]) by deleting the *Xba*I fragment that extends from the 3′ end of *hsp23* through the 3′-flanking sequence to the *Xba*I site in the polylinker (30).

The plasmid containing the *hsp26* gene is g26PB, and it was constructed by cloning the ~3-kb *Pst*I-*Bam*HI fragment spanning the *hsp26* gene from aDm202.7 (5) into *Pst*I-*Bam*HI-cut pGEM3 (27).

The plasmid containing the *hsp27* gene is pG3.27s, and it was constructed by cloning the ~1.5-kb *Pst*I-*Sal*I fragment containing the *hsp27* gene from aDm202.7 (5) into *Pst*I-*Sal*I-digested pGEM3 (30).

Nuclear run-on assays. *Drosophila* SL2 cells were grown at 22°C in Shields and Sang (S2) medium (13). To start an instantaneous heat shock, equal volumes of S2 medium at 56°C and uninduced cells at 22°C (ranging in density from 8×10^6 to ~1.3×10^7 cells per ml) were added to a spinner flask preheated to 37°C. The time of addition was taken to be time zero. At 30, 60, 90, 120, and 300 s after initiation of the heat shock, samples (~2×10^8 to 3×10^8 cells) were removed and rapidly mixed with ice-cold glycerol in a large glass beaker to give a final glycerol concentration of 7%. The sample was then immediately placed in an ice bucket containing liquid nitrogen, and at the same time liquid nitrogen was added to the cell sample in the beaker. It takes 10 to 12 s to remove the cell sample, mix it with glycerol, and add it to the liquid nitrogen. The recorded time point was the time when the sample was added to the liquid nitrogen. Within <10 s, the cell sample was fully frozen. Each sample was then stored at −70°C. The non-heat-shocked cells were also frozen and processed in the same way. To isolate nuclei, only one sample at a time was processed to minimize the processing time. Each sample was thawed, and nuclei were isolated as described previously (28).

Nuclear run-on assays and RNA hybridizations were carried out as described earlier (28), except that the run-on time was 1.5 or 2 min. To prepare the filters, approximately 3 μg of each restriction enzyme-digested plasmid DNA was electrophoresed on 1% agarose gels and transferred to a GeneScreen Plus membrane as described earlier (28).

Densitometry was also carried out as described elsewhere (24).

In vivo UV cross-linking. *Drosophila* SL2 cells were grown to a density of ~1×10^7/ml at 22°C. The instantaneous heat shock was applied as described above. At each time point a sample was removed (1.6×10^8 cells). The sample was then irradiated with five flashes from a Xenon lamp (Xenon Corp.) powered by a Xenon model 457 micropulser set at 10,000 V, with the duration of each flash being ~40 μs (11). Samples were removed from the spinner flask so that the time point would be the time of exposure to the first flash. It took approximately 10 s to deliver five flashes of light to each cell sample.

Samples were then processed as described previously (13), with the exception that Omnisorb cells (Calbiochem) were used instead of Pansorbin cells (Calbiochem) to precipitate immune complexes. To examine the distribution of polymerase on the *hsp70* genes, DNA-protein complexes from approximately 3×10^7 cells were removed and digested with *Xho*I and *Pst*I. To analyze the histone genes equal amounts of DNA-protein complexes were digested with *Pst*I. The eluted DNA was electrophoresed on a 1% agarose gel and transferred to a GeneScreen Plus membrane as described above.

To detect expression of the *H1* gene, the plasmid pUH1.9 (described above) was digested with *Pst*I, and the 615-bp fragment corresponding to the *H1* transcription region was purified. To detect expression of the core histone genes, a plasmid containing the *H2A* gene (described above) was digested with *Bam*HI and *Eco*RI to release a fragment containing the *H2A* transcription unit. To detect the *hsp70* gene, the plasmid p70X2.6 was digested with *Xho*I and *Bam*HI. This produces two fragments corresponding to the *hsp70* coding region: one from position −195 to +1252, and the other from +1252 to +2408. All fragments were individually purified from a 1% agarose gel. DNA was labelled by random priming (8).

Quantitation of RNA polymerase II molecules on the *hsp70* and *H1* genes. For nuclear run-on assays each filter contained the transcription units of the *hsp70*, *H1*, and core histone genes and, in some cases, the transcription units of the small heat shock genes. The fully induced *hsp70* gene contains approximately 30 transcribing polymerase molecules (24), or about 12.3 polymerases per kb (the transcription unit is ~2.4 kb). Within 300 s after initiation of heat shock the *hsp70* gene is fully induced (see below), so the number of transcribing polymerases on the gene at this time point is taken to be 30. The level of transcription of the core histone genes (*H2A*, *H2B*, *H3*, and *H4*) does not change dramatically between non-heat shock and full heat shock temperatures (7, 10). Using the core histone genes as an internal standard for each filter, we could estimate the number of polymerase molecules on the *hsp70* gene at each time point. The numbers of polymerases on the small heat shock genes were also estimated. Each of these genes is present as a single copy in the genome, and their sizes are taken to be 1.03, 0.96, 1.05, and 1.25 kb (*hsp22*, *hsp23*, *hsp26*, and *hsp27*, respectively) (4).

We were also able to quantitate the number of polymerase molecules on the *hsp70* gene by in vivo UV cross-linking (see below for a description of the technique). At 300 s the *hsp70* gene has a polymerase density of 1 molecule per ~80 bp (as the fully induced 2.4-kb gene has 30 elongating polymerases [see above]). Using this number, we calculated the number of polymerase molecules on the different fragments at each time point. For example, the 87A 5′-most fragment (1,369-bp band) (see Fig. 4A and B) should contain ~14.8 polymerase molecules when fully induced (1 polymerase per 80 bp; this fragment contains 1,184 bp of transcription coding region). For each time point the percentage of immunoprecipitation of this fragment was determined by comparing it with the band in the totals lane. By comparing the percentage of immunoprecipitation of this fragment at each time point with that of the fully induced gene (when this fragment contains ~14.8 polymerase molecules), we were able to determine directly the number of polymerase molecules on this fragment at each time point. A similar logic was applied to each of the remaining fragments.

RESULTS

Nuclear run-on assays can detect rapid changes in transcription after an instantaneous heat shock. Nuclear run-on assays provide one way of examining the distribution of transcriptionally engaged RNA polymerase molecules on a variety of

genes. We applied this technique to examine the distribution of RNA polymerase II on a series of *Drosophila* genes at short intervals after initiation of a heat shock response. *Drosophila* SL2 cells growing at 22°C (the non-heat shock temperature) were given an instantaneous heat shock by adding an equal volume of 56°C S2 medium, so that the overall temperature of the culture was brought to 37°C (the full heat shock temperature) in less than 5 s. At various times after this instantaneous heat shock we removed a sample of cells and quickly froze them in liquid nitrogen. After thawing the cells and isolating nuclei, we examined the distribution of RNA polymerase by incubating the nuclei in a transcription run-on buffer including [^{32}P]UTP. If the nuclear run-on time is very short (1.5 or 2 min), polymerases are not able to transcribe more than ~250 bp in vitro before the termination of the reaction (see below). Hybridizing the radiolabelled RNA to a filter containing restriction enzyme-cut plasmid DNA revealed the distribution of radiolabelled RNA, which approximates the in vivo distribution of RNA polymerase on the gene.

Figure 1A shows the genes examined by this method. The plasmid containing the *hsp70* gene was digested with the restriction enzymes *Ban*I and *Sca*I to divide the transcription region into four fragments. Each filter also contained restriction enzyme-digested DNA of the core histone genes and the *H1* gene. The transcription levels of the core histone genes do not change dramatically after heat shock (7, 10), while the level of *H1* mRNA is known to be reduced after heat shock (7).

Figure 1B shows the hybridization of radiolabelled run-on transcripts to *hsp70* and the histone genes. From these data, we estimated the number of RNA polymerase molecules on the *hsp70* gene as a function of the time after initiation of the heat shock treatment (see Materials and Methods) (Fig. 2A). In non-heat-shocked cells there is very little transcription throughout the body of the *hsp70* gene. A very low level of transcription corresponding to the 5′ fragment was seen; as this was confined to the 5′ fragment, it was presumably due to the escape of some paused polymerase into the body of the gene during the handling of the nuclei. It was not until between 30 and 60 s after the initiation of heat shock that an increase in the level of transcription occurring on the 5′ fragment was seen. At 60 s there was an average of 0.6 ± 0.2 elongating polymerase (or 2.3 ± 0.9 polymerases per kb) on the 5′ fragment of the gene. Since most of the transcription was confined to the 5′ fragment, polymerases did not transcribe more than ~250 bp during the nuclear run-on assay. Within 90 s polymerases were found predominantly on the first two fragments (fragment 5′ and fragment a), and by 120 s the first polymerases had reached the 3′ fragment. By 300 s (5 min) the *hsp70* gene appeared to be fully induced, as first, the density of polymerases throughout the gene was fairly uniform and, second, the density of polymerase on the gene remained fairly constant between 6 and 20 min of heat shock (40).

From the distribution of polymerase that was seen, we are able to conclude that the freezing process rapidly halts polymerase movement. First, at the earliest time points (30 and 60 s) polymerases are confined to the 5′ fragment. Second, if polymerases were able to elongate during the freezing process and nucleus isolation, then on the fully induced gene we would expect to detect a gradient in the density of transcribing polymerases that would increase from the 5′ to the 3′ end of the gene. This is not observed. Third, the distribution observed at early time points corresponds to that seen by an in vivo UV cross-linking technique (see below).

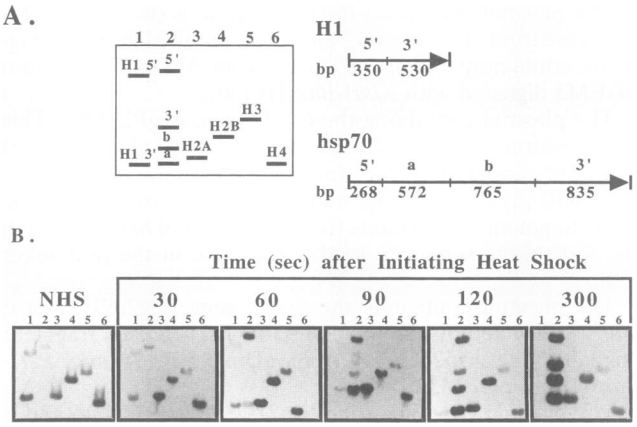

FIG. 1. Rapid changes in transcription of the *Drosophila hsp70* and histone *H1* genes after an instantaneous heat shock. (A) Key to Southern blots. Each filter contains the transcription coding region of the *hsp70*, *H1*, and core histone genes. Each of the plasmids containing the core histone genes was digested with *Eco*RI and *Hin*dIII to produce a fragment containing the entire transcribed region of each histone. The plasmid containing the *H1* gene was digested with *Eco*RI and *Hin*dIII, to produce two fragments containing the transcription unit. The sizes of the fragments are 594 bp for *H2A*, 815 bp for *H2B*, 1,100 bp for *H3*, 466 bp for *H4*, 1,210 bp for the 5′ fragment of *H1* (350 bp of transcription unit and the rest plasmid sequence), and 530 bp for the 3′ fragment. The plasmid containing the *hsp70* gene, p70X2.6, was digested with *Ban*I and *Sca*I to produce the indicated fragments (lane 2) (24). The 5′ end of the transcription unit is contained on a 1,437-bp fragment (268 bp of transcription unit and the rest plasmid vector sequence), fragment a is 572 bp, fragment b is 765 bp, and the 3′ fragment is 974 bp (835 bp of transcription unit and the rest plasmid vector sequence). The number of base pairs indicates the size of each fragment that is homologous to *hsp70* or *H1* RNAs; the heavy vertical lines indicate regions of transcription initiation and termination, whereas the lighter lines indicate restriction enzyme digestion sites. (B) Nuclear run-on assays. Cells were given an instantaneous heat shock as described in the text, and samples were removed at the indicated times and frozen in liquid nitrogen. The non-heat shock (NHS) sample was also frozen in this manner. After thawing of the cells and isolation of the nuclei, nuclear run-on assays generated radiolabelled RNAs that were hybridized to the Southern blots described for panel A. Lanes contain fragments as described for panel A. The bands seen in some of the *hsp70* lanes that are not of the indicated sizes are likely due to *Ban*I cutting inefficiently at two of its recognition sequences (24). As the levels of RNA hybridizing to these fragments are very low relative to the overall levels, they should not significantly affect our conclusions.

How quickly does the first wave of polymerase progress through the *hsp70* gene? By nuclear run-on assays, we did not observe transcription at the 5′ end of the gene until about 60 s after heat shock, and this wave of polymerase did not reach the 3′ fragment until 120 s after heat shock (Fig. 2A). This means that this first wave transcribed through 1.33 kb (fragments a and b in Fig. 1A) in about 60 s, giving a rate of ~1.3 kb/min.

Throughout the time course, the level of transcription of the core histone genes remained fairly constant. However, the level of radiolabelled RNA hybridizing to the *H1* gene decreased very rapidly, so that by 300 s it was very difficult to detect any nascent RNA transcripts corresponding to this gene (Fig. 1B). We were also able to calculate the decrease in transcription of the *H1* gene at each time point (Fig. 2B).

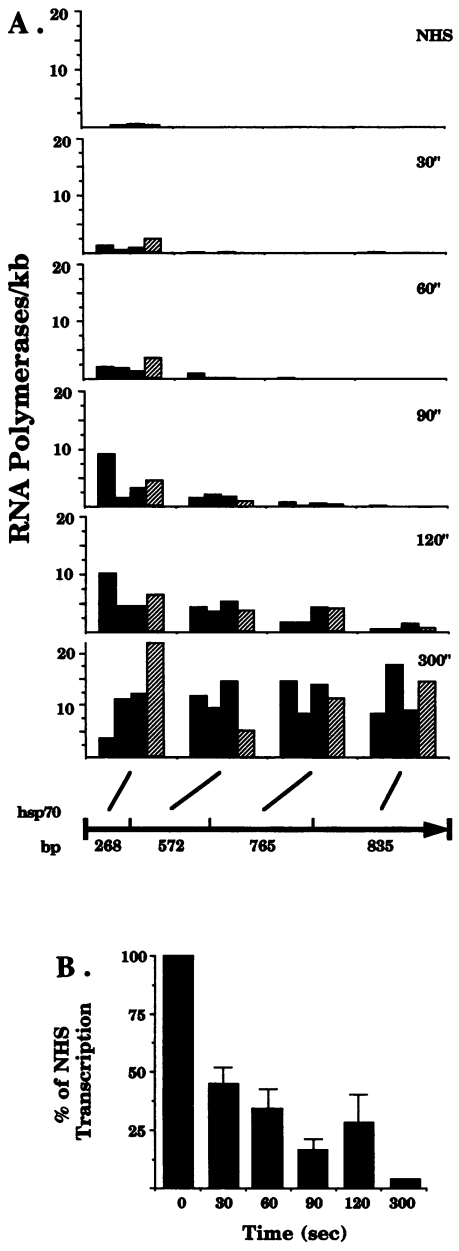

FIG. 2. Rapid changes in transcription of the *hsp70* and *H1* genes at 30-s intervals after initiation of an instantaneous heat shock. (A) *hsp70*. The number of polymerase molecules on each of the four transcription coding fragments is expressed as a density (number of polymerases per kilobase). Each set of bars represents an independent experiment (a total of four). Quantitation was carried out as described in Materials and Methods. In some cases the level of hybridization of radiolabelled RNA was too low to be accurately quantitated (bars are not shown). (B) Decrease in transcription of the *H1* gene. The level of transcription in the absence of heat shock (NHS) was taken to be 100%, and the level at each time point was determined relative to this initial level (with the core histone genes as an internal standard). These data are the averages of four independent experiments (± standard deviation), except for the 300-s time point (three experiments), for which only one value is shown, since in two of the three experiments the level of hybridization of radiolabelled RNA was too low to be accurately quantitated (values of <9% and <13%).

Transcription of this gene was reduced by ~50% within 30 s and continued to decrease so that by 300 s the level was less than 10% of that of the non-heat shock sample.

In vivo UV cross-linking shows that polymerase density on the histone *H1* gene is rapidly reduced by heat shock. The rapid reduction in transcription of the *H1* gene after heat shock, as seen in nuclear run-on assays, may be due to fewer polymerases transcribing the gene or to their density remaining constant but their rate of movement through the gene being lowered. To resolve this issue, we examined polymerase density on the *H1* and core histone genes directly by in vivo UV cross-linking. UV irradiation of SL2 cells covalently cross-links protein to DNA. These complexes can then be isolated by immunoprecipitation with an antibody directed against a specific protein, in this case RNA polymerase II. This technique has revealed the presence of an RNA polymerase II molecule at the 5′ end of the *hsp70*, *hsp26*, and β-*1 tubulin* genes (11, 29) and the distribution of polymerase on a series of *Drosophila* genes (10, 12). To examine polymerase distribution at early times after an instantaneous heat shock, the time of UV irradiation was reduced by using a Xenon flash lamp, so that cells were exposed to five closely spaced ~40-μs bursts of irradiation (11). After purification and restriction enzyme cutting of the protein-DNA complexes, an antibody directed against RNA polymerase II was used to immunoprecipitate DNA fragments that were cross-linked to RNA polymerase. The amount of each DNA fragment immunoprecipitated was quantitated by comparing it with a small fraction of the total amount of DNA that was present in the immunoprecipitation reaction (referred to below as the totals). The pattern of immunoprecipitated fragments relative to the totals represents the distribution of RNA polymerase at the time of UV irradiation.

After digestion of genomic DNA with *Pst*I, two main fragments containing the histone genes are produced, a 615-bp fragment that contains most of the *H1* transcription region and a 2.8-kb fragment that contains most of the transcription region for the core histones (Fig. 3A). On the *H1* gene, polymerase density was found to decrease rapidly after heat shock, and by 300 s the density was ~10% of that found in uninduced cells (Fig. 3B and C). Reprobing of this filter to detect transcription levels of the core histone genes revealed that the level of immunoprecipitation of the 2.8-kb fragment remains fairly constant as a function of time (Fig. 3B and C). Although this probe also detects some transcription from the 3′ end of the *H1* gene, the level was low enough that it should not interfere with our conclusions. We are unable to rule out the possibility that the conformation of polymerase on the *H1* gene has been modified by heat shock so that its efficiency of cross-linking to DNA is reduced; however, this is unlikely, considering that immunoprecipitation of polymerase on the core histone genes is unaffected by heat shock. These results therefore suggest that the reduction in transcription of *H1* observed in nuclear run-on assays is not simply due to a reduction in the rate of polymerase transcription but rather to the association of fewer RNA polymerase molecules with the *H1* gene.

In vivo UV cross-linking shows that polymerase density on the *hsp70* gene is rapidly increased by heat shock. We also examined the distribution of RNA polymerase II on the *hsp70* genes by in vivo UV cross-linking. There are five copies of the *hsp70* gene in SL2 cells; three are located at cytological position 87C, and two are located at 87A (14). When the purified protein-DNA complexes are digested with *Xho*I and *Pst*I, the patterns of bands corresponding to the

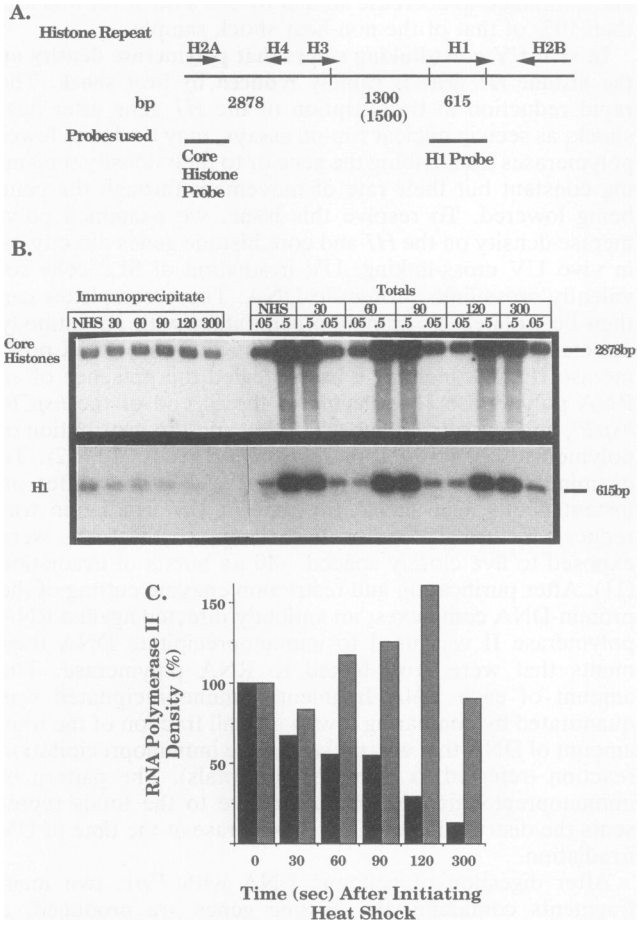

FIG. 3. Density of RNA polymerase II on the histone genes examined by in vivo UV cross-linking after an instantaneous heat shock. (A) Restriction map of one repeat of the histone locus (from reference 21). Each arrow represents a transcription unit and the direction of transcription. Vertical lines indicate the sites of *Pst*I digestion. The sizes of the fragments after digestion are presented in base pairs. The *H1* probe is the 615-bp *Pst*I fragment from the internal region of the gene (from +57 to +672). The probe used to detect the core histones is a 594-bp *Eco*RI-*Hin*dIII fragment containing the transcription unit of the *H2A* gene. There are two varieties of histone repeat, which differ by an insertion of 200 bp 3′ to the *H3* gene (12), producing a 1,300- or 1,500-bp band after *Pst*I digestion. Neither of these is detected by the probes used in this experiment. (B) Autoradiogram of filters containing restriction-digested immunoprecipitated DNA probed with fragments spanning either the *H1* or the *H2A* gene. For each sample the corresponding 0.5 and 0.05% of the totals are shown. The upper panel shows the hybridization pattern after the filter was probed with the *H2A* probe (3.5-h exposure). The lower panel shows the hybridization pattern after the filter was probed with the *H1* probe. The autoradiogram for the immunoprecipitated samples was exposed for 24 h, while that shown for the totals was exposed for only 4 h. NHS, non-heat shock. The time after heat shock initiation is given in seconds. (C) Polymerase density on the histone genes. The percentage of immunoprecipitation of the fragment containing the *H1* gene (filled bars) and the core histone genes (shaded bars) was determined for each time point by comparing it with the corresponding fragment in the totals lane. The polymerase density on *H1* and the core histone genes in uninduced cells was assumed to be 100%, and the density of polymerase on these genes at each time is expressed as a percentage of the initial density.

hsp70 genes differ for genes contained at 87A and 87C because of small differences in the sequences of *hsp70* at the two loci (Fig. 4A). By quantitating the amount of each fragment of *hsp70* that was immunoprecipitated, we were able to calculate the density of polymerases on each fragment (see Materials and Methods).

The induction response was similar to that seen in nuclear run-on assays (Fig. 1B). In uninduced cells, a paused polymerase is located between positions +17 and +46 (9, 26, 28). Here, as expected, we observed that polymerase cross-linked to a fragment that contains this region (at 87A, the 1,369-bp band in Fig. 4A) but not to a fragment that does not (at 87C, the 1,092-bp band spanning from +85 to +1177 in Fig. 4A). Polymerase density increased significantly on the 87A 5′ fragment after 30 to 60 s. As this increase was found on the 87A 1,369-bp fragment and not on the 87C 1,092-bp fragment (containing sequences from +85) (Fig. 4B and C), it indicates that most of the polymerases elongated from the pause site but did not progress further than +85. By 90 s polymerases passed the +85 site and were found on the 1,092-bp 87C fragment. A reprobing of this filter with sequences corresponding to the 3′ end of the gene showed that a significant level of polymerase was not detected on the 3′-most fragments until between 90 and 120 s (Fig. 4B and C).

The rate of movement of polymerase can also be estimated. On the 87A copies, polymerase density on the 5′ fragment does not begin to increase until 60 s, and no increase on the 3′ fragment is seen until 120 s. This means that polymerase has transcribed through the first 1,184 bp (contained within the 1,369-bp band in Fig. 4B) in 60 s, a rate of ~1.2 kb/min (Fig. 4B and C). Polymerase density did not increase on the 1,092-bp fragment of the 87C *hsp70* genes until 60 s, and no increase in polymerase density was detected on the 3′ fragments until ~120 s, which means that these polymerases transcribed through the 1,092-bp fragment in ~60 s, a rate of 1.1 kb/min (Fig. 4B and C). Therefore, there is little difference between the rates of movement of the first wave of polymerase through the 87A copies and the 87C copies of *hsp70*. The average of these two estimates from in vivo UV cross-linking is ~1.1 kb/min.

Induction of the small heat shock genes *hsp22*, *hsp23*, *hsp26*, and *hsp27*. We also examined transcription levels on the small heat shock genes by nuclear run-on assays (Fig. 5). As these genes are small relative to *hsp70*, it is difficult to see a polarity of transcription from the 5′ to the 3′ end at early times of induction. We did not attempt to examine the kinetics of polymerase movement but simply showed that these genes are also rapidly induced. Induction of all these genes was detected by 90 s after heat shock, and by 300 s each gene had a density of RNA polymerases similar to that of *hsp70* (compare Fig. 2A and Fig. 5).

DISCUSSION

The uninduced *hsp70*, *hsp26*, *hsp23*, and *hsp27* genes appear poised for a rapid transcriptional response. These promoters already contain an RNA polymerase II molecule that has initiated transcription but has paused after transcribing 17 to 46 bases (9, 26, 28, 29). Moreover, the chromatin structure containing these promoters is in an open configuration (2, 5, 41), allowing HSF ready access to the HSEs.

The kinetics of the transcriptional induction of the major heat shock genes was examined here both by nuclear run-on assays and by in vivo UV cross-linking. Although very little increase in transcription of *hsp70* was seen after 30 s of heat

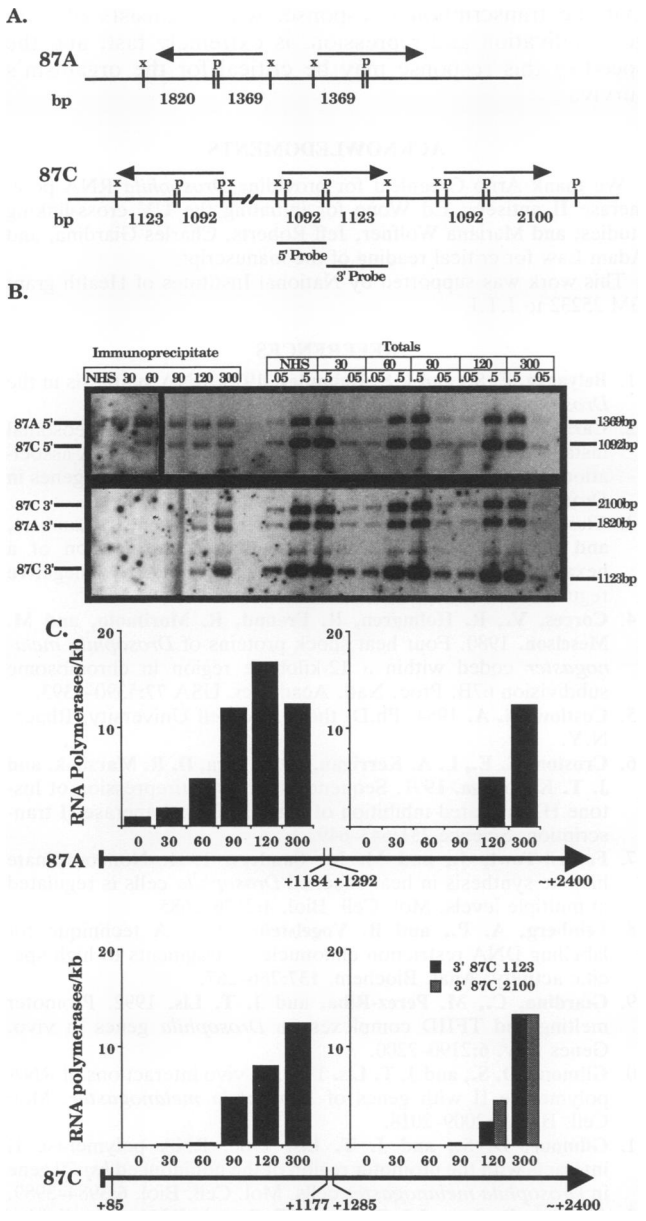

FIG. 4. Distribution of RNA polymerase II on the *hsp70* genes examined by in vivo UV cross-linking after an instantaneous heat shock. (A) Restriction map of the *hsp70* locus in *Drosophila melanogaster* (from references 11 and 22). Arrows indicate each transcription unit and the direction of transcription. Digestion of the UV cross-linked protein-DNA complexes with *Xho*I (x) and *Pst*I (p) produces the indicated fragments. The two probes used are an *Xho*I-*Bam*HI fragment from −195 to +1252 and an *Xho*I-*Bam*HI fragment from +1252 to +2408. The 5′ probe only hybridizes to the 1,369- and 1,092-bp bands from 87A and 87C, respectively. The 3′ probe hybridizes to the 1,820-bp band from 87A and the 1,123- and 2,100-bp bands from 87C. (B) Autoradiograms of filters containing restriction enzyme-digested immunoprecipitated DNA probed with the 5′ fragment and then reprobed with the 3′ fragment. The upper panel shows the hybridization pattern after the filter was probed with the 5′ probe; the autoradiogram shown was exposed for 13 h, except for the non-heat shock (NHS) 30- and 60-s samples, for which the exposure was 64 h. The lower panel shows the hybridization pattern after the filter was reprobed with the 3′ probe (51-h exposure). For each sample the corresponding 0.5 and 0.05% of the totals are shown. The bands corresponding to the fragments expected from the restriction enzyme digestion are indicated. (C) Quantitation of RNA polymerase II density on the *hsp70* genes. Arrows represent the *hsp70* transcription unit, heavy vertical lines represent the regions of transcription initiation, and arrowheads represent the region of termination. Restriction enzyme digestion sites are shown (in base pairs) with respect to the transcription initiation site. Quantitation was carried out as described in Materials and Methods. Density of polymerase molecules (polymerases per kilobase) on each fragment is shown. Time after heat shock initiation is given in seconds.

shock, transcription can be detected on the 5′ fragment within 60 s. Thus, within 1 min the *hsp70* promoter becomes transcriptionally active. As for *hsp70*, induction of the small heat shock genes (*hsp22*, *hsp23*, *hsp26*, and *hsp27*) occurs rapidly after heat shock. The fact that these genes are all rapidly induced with kinetics similar to that of the *hsp70* gene demonstrates that the induction of the main heat shock genes occurs in a highly coordinate manner.

Drosophila HSF is found in nuclei of uninduced cells (38), which may help to explain the rapid activation that we observe here. After heat shock HSF binds to HSEs located upstream of heat shock genes and activates transcription (3, 35, 39, 42). This ability to activate transcription is believed to occur in two steps. First, HSF must multimerize to form a trimer that is then able to bind tightly and specifically to HSEs (38). HSF is known to be capable of binding to HSEs as early as 30 s after initiation of a heat shock (43), which is rapid enough to explain our observation of transcription of the *hsp70* gene beginning to occur sometime between 30 and 60 s. Second, it is possible that HSF must also be phosphorylated before it can activate transcription, since in mammalian and yeast cells HSF becomes phosphorylated after heat shock (15, 32–34). Once HSF binds to its target sequences upstream of *hsp70*, it must stimulate the paused polymerase to resume elongation. Since pausing of polymerase remains the rate-limiting step in transcription during heat shock (9, 24), the second polymerase must be recruited to the promoter, initiate transcription, and elongate to the pause region. Therefore, it appears that all of these events occur within the first 60 s of heat shock.

A similar rapid response to heat shock appears to occur for yeast heat shock genes. The nucleosomal structure along the *hsp82* gene was found to be disrupted (as assayed by DNaseI digestion) within 50 s of initiation of heat shock, although the first full-length transcripts were not found until after 100 s (17). From the time it takes to generate the first detectable transcript, one can calculate a minimum polymerase movement of ~1.4 kb/min, since a polymerase takes 100 s to traverse the 2.3-kb gene. This agrees with the rate of movement of polymerase we observed here for the *Drosophila hsp70* gene.

We estimate that the first wave of polymerase transcribes through the *hsp70* gene at an elongation rate of ~1.2 kb/min. Thummel et al. estimated the in vivo rate of polymerase to be ~1.1 kb/min on the *Drosophila E74* gene (36). More recently, the rate of transcription has also been calculated to be about 1.4 kb/min on the *Drosophila Ubx* gene (31). Thus, our estimate of polymerase movement through the *hsp70* gene at ~1.2 kb/min is similar to other in vivo estimates.

In contrast to the rapid activation of heat shock genes, transcription of the *H1* gene is rapidly repressed. We estimate from nuclear run-on assays that the density of polymerases on the histone *H1* gene decreases to ~50% within 30 s of initiation of heat shock and decreases further to ~10% within 300 s. In vivo UV cross-linking results indicate that

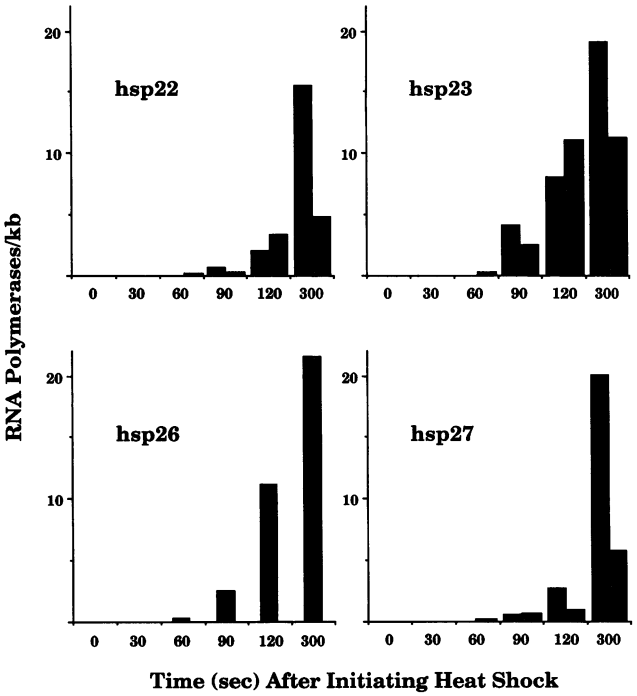

FIG. 5. Density of transcribing RNA polymerase II molecules on the small heat shock genes at 30-s intervals after initiation of an instantaneous heat shock, as determined by nuclear run-on assays. These results are from two independent experiments for *hsp22*, *hsp23*, and *hsp27* but from only one for *hsp26*. Quantitation was carried out as described in Materials and Methods.

this decrease in transcription is not because polymerases on this gene have a reduced elongation rate but rather because there are fewer polymerases associated with the *H1* gene.

Why should the *H1* gene be turned off so rapidly? Histone *H1* has recently been shown to be capable of acting as a general repressor of transcription, especially at high ratios of *H1* to DNA (6, 16). After heat shock, DNA synthesis has been shown to be reduced. In mammalian cells a short heat shock is sufficient to arrest cell division at S phase (37). It is possible that continued production of histone *H1* during heat shock could have the effect of globally repressing gene transcription. Thus, it might be important for a cell to cease production of histone *H1* as rapidly as possible after heat shock to ensure a return to a normal transcription pattern upon recovery from heat shock.

What causes the rapid turnoff of the histone *H1* gene? RNA polymerase itself is unlikely to be affected, since it is still capable of transcribing the core histone genes efficiently. It was recently proposed by Westwood and colleagues (38) that HSF itself can act as a repressor of gene expression. Localization of HSF by in situ immunofluorescence to *Drosophila* polytene chromosomes demonstrated that HSF can be found at as many as 156 individual sites (38), one of which is in the region of the histone genes (region 39D). It is intriguing that repression of the *H1* gene is detected even more rapidly than induction of the major heat shock genes. Perhaps the kinetics of HSF activation and repression are different for the two processes, or alternatively, the repression of the *H1* gene may be unconnected to HSF activity.

Induction of heat shock genes after exposure to heat shock is designed to protect the organism and aid in its recovery (for a review, see reference 23). From our results it is clear that the transcriptional response, which consists of both gene activation and repression, is extremely fast, and the speed of this response may be critical for the organism's survival.

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