Regulated Transcription of the Genes for Actin and Heat-shock Proteins in Cultured Drosophila Cells

R. CRAIG FINDLY and THORU PEDERSON
Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

ABSTRACT
The transcription of three specific genes has been examined in heat-shocked Drosophila cells by hybridizing pulse-labeled nuclear RNA with cloned DNA sequences. Actin gene transcription is rapidly and profoundly suppressed upon heat shock but returns to near-normal levels after cells are placed back at their normal culture temperature (25°C). Conversely, the transcription of genes coding for 70,000- and 26,000-dalton heat-shock proteins increases dramatically and with extraordinary rapidity (60 s) after heat shock. The temporal patterns of 70,000- and 26,000-dalton heat-shock gene transcription are nearly superimposable, indicating that, although they are not closely linked cytologically, these genes are nevertheless tightly coregulated. The abundance of heat-shock gene transcripts reaches remarkable levels, e.g., 70,000-dalton heat-shock gene transcripts account for 2–3% of the nuclear RNA labeled during the first 30 min of heat shock. When heat-shocked cells are returned to 25°C, the rates of transcription of the heat-shock genes fall back to the low levels characteristic of untreated cells. To confirm the low level of heat-shock gene transcription in normal cells, nuclear RNA was purified from unlabeled (and otherwise unhandled) 25°C cells, end-labeled in vitro with 32P, and hybridized to cloned heat-shock DNA sequences. These and other data establish that the genes for 70,000- and 26,000-dalton heat-shock proteins in cultured Drosophila cells are active at 25°C, and that their rate of transcription is greatly accelerated upon heat shock rather than being activated from a true “off” state. The rapidity, magnitude, and reversibility of the shifts in actin and heat-shock gene transcription constitute compelling advantages for the use of cultured Drosophila cells in studying the transcriptional regulation of eukaryotic genes, including one related to the cytoskeleton.

A specific set of puffs is rapidly induced by heat shock in the polytene chromosomes of Drosophila larval salivary glands (25). This is accompanied by the synthesis of a small number of new polypeptides, the heat-shock proteins, and the cessation of most other translation in the cell (30). These initial observations have led to extensive studies of the chromosomal localization and DNA sequence organization of the genes directing the heat shock response, as well as analyses of messenger RNA and protein synthesis during heat shock (1). It now appears that the Drosophila heat-shock response may be an example of a more general biological reaction to environmental stress because inducible genes coding for sets of proteins of molecular weights similar to those of the Drosophila heat-shock proteins have been identified in a wide phyletic range of eukaryotes besides insects, including yeast, slime mold, and mammalian cells (10, 14, 22).

In Drosophila, the synthesis of heat-shock proteins is associated with the appearance of newly synthesized messenger RNA’s in polyribosomes (19, 20, 28, 29). However, it is not known whether this reflects transcriptional activation of the heat-shock genes, alterations in posttranscriptional processing of nuclear RNA’s, or both. In the present study, we have used cloned Drosophila DNA probes to investigate directly the transcription of two genes coding for heat-shock proteins. In addition, we have used a cloned Drosophila actin gene to examine the transcription pattern of a non-heat-shock gene under these same conditions. We find that the transcription of heat-shock genes is activated immediately and dramatically upon heat shock, whereas the transcription of actin genes is rapidly but reversibly shut off.

MATERIALS AND METHODS

Cell Culture
Drosophila melanogaster tissue culture cells (Schneider line 2—obtained from Robert Freund) were propagated in 75-ml Falcon flasks at 25°C in 30 ml of Schneider’s medium (Grand Island Biological Co., Grand Island, N. Y. [GIBCO]) supplemented with 15% heat-inactivated fetal calf serum and 5 mg/ml Bacto-Peptone (Difco Laboratories, Detroit, Mich.). Cultures were split 1:5 every...
5 d. For large-scale experiments, cells were grown in suspension culture at 25°C. Approximately 75 ml of cells from 5-d monolayer cultures were inoculated into 300 ml of supplemented Schneider’s medium in a 2-liter flask and stirred gently. Antibiotic-antimycotic mixture (GIBCO) was added to 1%. The cell population doubling time of these suspension cultures was ~24 h. When the cultures had reached a density of 5–10 x 10⁵ cells/ml, the cells were harvested by centrifugation at 1,500 rpm for 4 min in an IEC FR-1 centrifuge (International Equipment Co., Danvers Corp., Needham, Mass.)

**Heat Shock and Labeling Conditions**

Suspension cultures were incubated for 1 h at 25°C with 5-fluorouridine (5 μg/ml), which selectively inhibits RNA synthesis in cultured Drosophila cells (15). The cells were then collected by low-speed centrifugation, resuspended in the same medium containing 5-fluorouridine at a three- to fivefold higher cell concentration, and incubated at 25°C for 30 min before further handling. To heat shock the cells, cultures were placed in a 50°C water bath with stirring. When the temperature of the culture reached 34°C (~30 s), the cells were rapidly transferred to a 35°C circulating water bath and then pulsed-labeled with [³H]uridine (15–50 μCi/ml) for the desired period of time.

**Cell Fractionation**

All steps were performed at 4°C or on ice. Cells were diluted into 100 mM NaCl, 10 mM CaCl₂, 10 mM Tris-HCl, pH 7.2, collected by centrifugation, and washed extensively in 10 vol of lysozyme buffer (LB). LB contains a ribonuclease inhibitor prepared from HeLa cells as follows. HeLa cells were grown as previously described (23), washed in balanced salt solution (8), and resuspended in 10 vol of 30 mM NaCl, 10 mM CaCl₂, 100 mM Tris-HCl, pH 8.5. The detergent Nonidet P-40 was added to 0.5% (vol/vol) and the cells were lysed by vortexing. In other cases, the cells were disrupted by Dounce homogenization (Kontes Co., Vineland, N. J.) in 10 mM NaCl, 1.5 mM MgCl₂, 10 mM Tris-HCl, pH 7.0 (reticulocyte standard buffer [RSB]). In both cases, the nuclei were pelleted by centrifugation and the cytoplasmic fraction was centrifuged in a Beckman Spinco 50Ti fixed angle rotor (Beckman Instruments, Inc., Fullerton, Calif.) at 40,000 rpm for 1 h (4°C). The postmicrosomal supernate was recovered and stored at ~70°C. Cytoplasmic extracts of HeLa cells prepared in this manner are potent inhibitors of Drosophila ribonucleases, as shown by their effects on the recovery of high molecular weight Drosophila RNA (R. Findly and T. Pearson, unpublished results). Similar RNAse inhibitors are present in cytoplasmic extracts from rat liver (26) and human placenta (3).

Brief vortexing of Drosophila cells in LB resulted in complete lysis, as monitored by phase-contrast microscopy. Nuclei were centrifuged at 2,000 g for 4 min, and resuspended in 10 vol of RSB containing HeLa cytoplasmic RNAse inhibitor (see above). The nuclei were centrifuged, washed again in 10 vol of RSB without inhibitor, and then resuspended in a small volume of RSB. The yield of nuclei at this stage was 75–80%.

**Isolation of Nuclear RNA**

Nuclei in RSB were incubated with pancreatic DNase ( Worthington Biochemical Corp., Freehold, N. J.) at 50 μg/ml for 30 s at 37°C. The DNAse was purified free of RNAse before use by affinity chromatography on agarose–5’-(4-aminoethylphosphoryl-uridine)-2’(3’)-phosphate (18). SDS was then added to 0.5% and EDTA to 25 mM, followed by an equal volume of phenol:chloroform:isoamyl alcohol (99:1). The combined organic phases were also 0.5% and EDTA to 25 mM, followed by an equal volume of phenol:chloroform:isoamyl alcohol (99:1). The combined organic phases were also washed extensively in 2x SSC, and incubated with 201xg/ml pancreatic RNAse for 1 h at 37°C. The filters then were washed in 2x SSC, then in 2x SSC containing 0.5% SDS, in 2x SSC again, followed by cold 5% trichloroacetic acid for 5 min, rinsed twice in 70% ethanol, dried, and counted in toluene-based scintillation fluid. After hybridization, filters were removed, washed extensively in 2x SSC and incubated with 20 μg/ml pancreatic RNAse for 1 h at 37°C. The filters then were washed in 2x SSC, then in 2x SSC containing 0.5% SDS, in 2x SSC again, followed by cold 5% trichloroacetic acid for 5 min, rinsed twice in 70% ethanol, dried, and counted.

A filter containing only the pBR322 vector DNA was included in all hybridizations. The extent of RNA hybridization with the recombinant plasmid DNA was determined by subtracting the cpm on the pBR322 filter from that on the cloned Drosophila DNA filters. Radioactivity on the pBR322 filters ranged from 50 to 100 cpm over instrument background. Hybridization reactions were challenged with a second set of filters after the initial hybridization. No additional hybridization was observed with pJ1B or pDmA2, but in some instances there was a further 10–20% hybridization with pWP232.1 DNA. In these cases, the amount of [³H] radioactivity hybridized in the first and second reactions was summed. In all cases, the amount of RNAse-resistant [³H] radioactivity hybridizing with pWP232.1 DNA was corrected for the fact that the plasmid contains only 1 kb of the total 70,000-dalton heat-shock protein gene (2.4 kb). No such corrections were made for pJ1B or pDmA2.

**Cloned DNA**

pWP232.1 (a gift of Ken Livak and Matthew Meselson, Harvard University) contains approximately the 5’ half of a 70,000-dalton heat-shock protein gene from D. melanogaster inserted into the plasmid pBR322 (16). pJ1B (a gift of Sam Wadsworth, Worcester Foundation for Experimental Biology) is a subclone of pJ1 (31), and contains the entire coding region for a 26,000-dalton heat-shock protein from D. melanogaster inserted into pBR322. pDmA2 (a gift of Eric Fyrberg and Norman Davidson, California Institute of Technology) includes most of the coding region for a Drosophila actin gene. pDmA2 contains 1.6 and 1.8 kilobase (kb) Hind III fragments derived from a partial Hind III digestion of the genomic Drosophila clone ADmA2 (11), inserted into pBR322. In all cases, plasmid DNA was isolated by ethidium bromide-CsCl banding of cleared lysates of bacteria that had been cultured ~16 h with chloramphenicol (6). Ethidium bromide was removed from CsCl-banded plasmid DNA by isopropanol extraction. The DNA was then purified from low molecular weight RNA by gel filtration on Biogel A15m in 1 mM EDTA, 10 mM Tris-HCl, pH 7.0 (12). The growth and extraction of recombinant plasmids was performed under F2 and HEK1 containment, as specified by the National Institutes of Health guidelines in operation during this investigation.

**DNA-RNA Hybridization**

Plasmid DNA (2.5 μg) was loaded onto 24-mm nitrocellulose filters (Millipore-HA, Millipore Corp., Bedford, Mass. 01730) as previously described (21). Hybridizations were carried out in a final volume of 1 ml, using 10–50 μg RNA/ml in 4x SSC (1x SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), 0.2% SDS, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.2% bovine serum albumin (7) at 42°C for 60–70 h.

Total [³H] radioactivity in the hybridization mix was determined by spotting duplicate aliquots on nitrocellulose filters, which were then washed in cold 5% trichloroacetic acid for 5 min, rinsed twice in 70% ethanol, dried, and counted in toluene-based scintillation fluid. After hybridization, filters were removed, washed extensively in 2x SSC, and incubated with 20 μg/ml pancreatic RNAse for 1 h at 37°C. The filters then were washed in 2x SSC, then in 2x SSC containing 0.5% SDS, in 2x SSC again, followed by cold 5% trichloroacetic acid for 5 min, rinsed twice in 70% ethanol, dried, and counted.

A filter containing only the pBR322 vector DNA was included in all hybridizations. The extent of RNA hybridization with the recombinant plasmid DNA was determined by subtracting the cpm on the pBR322 filter from that on the cloned Drosophila DNA filters. Radioactivity on the pBR322 filters ranged from 50 to 100 cpm over instrument background. Hybridization reactions were challenged with a second set of filters after the initial hybridization. No additional hybridization was observed with pJ1B or pDmA2, but in some instances there was a further 10–20% hybridization with pWP232.1 DNA. In these cases, the amount of RNAse-resistant [³H] radioactivity hybridizing with pWP232.1 DNA was corrected for the fact that the plasmid contains only 1 kb of the total 70,000-dalton heat-shock protein gene (2.4 kb). No such corrections were made for pJ1B or pDmA2.

**RESULTS**

**RNA Synthesis after Heat Shock**

Fig. 1 shows the continuous [³H]uridine labeling kinetics of total cellular RNA in cultured Drosophila cells at their normal temperature of 25°C and also under conditions of heat shock (35°C). It can be seen that the incorporation of precursor into RNA is reduced at 35°C, indicating a general inhibition of transcription during heat shock. Additional experiments with cells pulse-labeled for 5 min at various times confirmed that transcription is gradually reduced after heat shock (not shown), in agreement with the results of Carlson and Pettijohn (5). However, the actual extent of transcriptional inhibition during heat shock cannot be quantitatively ascertained from these data because it remains possible that an expansion of the intracellular UTP pool could conceivably contribute to the decreased incorporation observed at 35°C. Therefore, in the following analyses of specific gene transcription, all data are presented as the percent of total labeled RNA found in hybrid. If the rate of transcription of a particular gene changes to either a greater or lesser extent than the overall rate of RNA synthesis,
then it follows that this will be detected as a change in the percent of total RNA hybridized. Thus, the results to be described represent preferential effects of heat shock on the transcription of specific genes, relative to RNA synthesis as a whole.

**Actin Gene Transcription is Rapidly Suppressed during Heat Shock**

Hybridization of 5 min pulse-labeled nuclear RNA with cloned pDmA2 DNA revealed that actin gene transcription is rapidly and almost completely shut off during heat shock (Fig. 2). After a 5-min pulse of [3H]uridine at the normal culture temperature of 25°C, 0.1% of the labeled nuclear RNA hybridizes to pDmA2 DNA, showing that actin gene transcripts are a highly prevalent component of the nuclear RNA in these cells. This level of hybridization is set at 100% in Fig. 2. It can be seen that within 10 min after heat shock, actin gene transcription has fallen to only ~5% of the 25°C value and remains at the 5–15% level throughout the 60-min period analyzed. In other experiments, actin gene transcription was found to remain suppressed at the low levels shown in Fig. 2 for as long as 3 h at 35°C.

Despite its intensity (Fig. 2), the inhibition of actin gene transcription imposed during heat shock is reversible. When cells heat-shocked for 30 min are returned to 25°C for 2 h and then analyzed, the level of actin gene transcription is observed to have returned to ~55% of that detected initially at 25°C ("25°C reversal" Fig. 2). This reactivation of actin genes suggests that heat shock does not irreversibly damage the regulatory capacity of the cell's transcription machinery.

**Transcription Patterns of Heat-shock Genes**

The transcription of heat-shock genes was investigated by hybridizing pulse-labeled nuclear RNA with cloned pPW232.1 and pJ1B DNA as probes for 70,000- and 26,000-dalton heat-shock protein genes, respectively. As shown in Fig. 3, 70,000-dalton heat-shock protein gene transcription is activated immediately after heat shock and remains at a relatively high level over the 60-min period examined. This is a rather stable, long-term effect inasmuch as values similar to those recorded at 10–60 min in Fig. 2 were also observed after 2 or 3 h of heat shock (not shown). The "overshoot" seen at 5 min after heat shock (Fig. 3) was observed consistently, although its significance is not clear.
The rapidity and intensity of this transcriptional activation is noteworthy. During the first 5 min of heat shock, when total RNA synthesis is declining (data not shown), 70,000-dalton heat-shock protein gene transcription is accelerated to such a great extent that these transcripts become 25 times more prevalent constituents of the total labeled nuclear RNA, increasing from 0.045% at 25°C to 1.28% within 5 min of heat shock (see legend, Fig. 3).

To examine the rapidity of the heat-shock gene activation phenomenon in greater detail, cells were pulse-labeled with \(^3\)H]uridine during the first 60 s after heat shock. This revealed that 2.2% of the labeled nuclear RNA hybridized to pPW232.1. The extreme rapidity of this effect indicates that it involves an actual transcriptional activation of 70,000-dalton heat-shock genes. The alternative possibility is that the 70,000-dalton heat-shock genes are actually transcribed at equal rates at 25° and 35°C, but that the transcripts are degraded almost instantly at 25°C. While not excluded by the data, this possibility seems remote because it is improbable that a cessation of nascent transcript degradation upon heat shock could lead to such a dramatic accumulation of 70,000-dalton transcripts within only 60 s without a corresponding increased rate of transcription.

Fig. 4 shows the activation time-course of a second gene, coding for a 26,000-dalton heat-shock protein (31). It can be seen that, both in its rapidity and intensity (>10-fold), this activation closely parallels that of the 70,000-dalton heat-shock protein genes (Fig. 3). In fact, the curves shown in Figs. 3 and 4 are almost superimposable, especially during the first 20 min of heat shock. Moreover, like 70,000-dalton heat-shock protein gene transcription, the level of 26,000-dalton heat-shock protein gene transcription was found to be sustained at the levels shown at 15–60 min in Fig. 4 even after 3 h of heat shock (data not shown). In addition, the activation of 26,000-dalton protein gene transcription was found to occur within 60 s of heat shock, as is the case for activation of 70,000-dalton protein gene transcription. Thus, in all respects examined, the temporal pattern of transcriptional activation of these two heat-shock genes is identical, indicating that they are tightly coregulated.

It was noted earlier that the inhibition of actin gene transcription during heat shock is reversible (Fig. 2). This is also true of the activation of the heat-shock genes. In Figs. 3 and 4, it can be seen that if cells are heat shocked for 30 min and then returned to 25°C for 2 h (25°C reversal), the level of both 70,000- and 26,000-dalton heat-shock protein gene transcription returns to the low levels characteristic of untreated cells. However, this reversal of 70,000- and 26,000-dalton gene transcription is not immediate. For example, if cells are subjected to a 30-min heat shock and then returned to 25°C for only 10 min, the levels of 5-min pulse-labeled nuclear RNA hybridizing to 70,000- or 26,000-dalton heat-shock DNA are identical to the values for 60 min at 35°C shown in Figs. 3, and 4, respectively. Thus, although the activation of these genes upon heat shock is remarkably rapid (see above), their inactivation following return of cells to 25°C appears to be a much more gradual process.

We wish to emphasize that the transcriptional activation of the 70,000- and 26,000-dalton heat-shock protein genes does not lead to a progressive increase in the nuclear abundance of these transcripts beyond the dramatic burst seen during the first few minutes of heat shock in Figs. 3 and 4. The fraction of labeled nuclear RNA in 70,000- or 26,000-dalton heat-shock protein gene transcripts remains relatively constant from 15 to 60 min after heat shock (Figs. 3 and 4), even though total RNA synthesis is decreasing during this period (not shown). This indicates that although the heat-shock genes are initially subjected to a dramatic activation, they then fall under the general inhibition of transcription imposed by heat shock. Yet, there is reason to believe that the heat-shock gene transcripts are special, for if cells are labeled for 30 min at 35°C, rather than 5 min as in Figs. 3 and 4, the percent of total nuclear labeled RNA hybridizable to the 70,000- and 26,000-dalton gene probes increases two to threefold (Table I). This raises the possibility that these transcripts may have somewhat longer half-lives and/or nuclear residence times than total nuclear RNA.

**Heat-shock Gene Transcription Confirmed in 25°C Cells**

The detection of a low but finite level of heat-shock gene transcription in untreated cells growing at 25°C is of interest (Figs. 3 and 4). One possibility is that these genes have a constitutive level of transcription in the absence of heat shock. Another possible explanation is that these genes are very sensitive to environmental perturbations and are merely activated by the 1-h 5-fluorouridine treatment and/or \(^3\)H]uridine pulse-labeling procedures. To examine this important point, nuclear RNA was extracted from unhandled 25°C cells that were neither treated with 5-fluorouridine nor pulse-labeled. The purified nuclear RNA was then labeled in vivo with \(^32\)P and assayed for 70,000- and 26,000-dalton heat-shock gene transcripts by hybridization with pPW232.1 and pJ1B DNA, respectively. As shown in Table II, an average of 0.025% of the nuclear RNA hybridized to pPW232.1 DNA and 0.018% hy-

![Figure 4](image-url)  
**Figure 4**  
Activation of 26,000-dalton heat-shock protein gene transcription. Nuclear RNA from cells pulse-labeled for 5 min as in Figs. 2 and 3 was hybridized with pJ1B DNA. Normalization of hybridization data to percent of maximum hybridization was as described in Figs. 2 and 3, using as 100% the values at 5 min of heat shock, which were 0.085% and 0.024% of the total nuclear RNA, respectively, in two separate experiments. These hybridization values corresponded to actual \(^3\)H radioactivities of between 250 and 450 cpm above that on pBR322 filters. 25°C reversal was as detailed in Fig. 2.

**Table I**  
Relative Stability of Heat-shock Gene Transcripts

| Transcript   | After 5 min | After 30 min |
|--------------|-------------|--------------|
| pPW232.1 (70,000 daltons) | 1.28 | 2.66 |
| pJ1B (26,000 daltons) | 0.06 | 0.20 |

* Labeled from 0 to 5 min after heat shock.
† Labeled from 0 to 30 min after heat shock.
Cells at 25°C were pulse-labeled in vivo for 5 min with \([3H]\)uridine. Gene transcripts can be detected, whereas after only 60 s at extraordinary rapidity when Drosophila cultured cells are exposed to heat shock, protein genes increase dramatically and the transcription of the genes coding for 70,000- and 26,000-dalton heat-shock proteins is increased. The transcription of the genes coding for 70,000- and 26,000-dalton heat-shock genes occurs at a low level in Drosophila cultured cells that have neither been heat shocked nor handled in any other way. Therefore, it appears that the transcriptional activation of Drosophila heat-shock genes described in this study does not represent a true "off-on" situation, but rather a dramatic increase in the rate of transcription of a set of already-active genes.

**DISCUSSION**

**The Heat-shock Response Involves Transcriptional Activation**

In the present investigation, the heat-shock response in Drosophila cultured cells has been directly examined at the level of gene transcription. The central finding is that the rate of transcription of the genes coding for 70,000- and 26,000-dalton heat-shock proteins increases dramatically and with extraordinary rapidity when Drosophila cultured cells are exposed to heat shock. For example, after a 5-min pulse label at 25°C, only a low level of 70,000-dalton heat-shock gene transcripts can be detected, whereas after only 60 s at 35°C, a remarkable 2.2% of the labeled nuclear RNA is 70,000-dalton gene transcript. Although this does not exclude the possible involvement of posttranscriptional or translational changes in bringing about the heat-shock response as defined at the level of protein synthesis, the present results demonstrate that a dramatic activation of specific gene transcription occurs in the Drosophila heat-shock response.

It could be argued that the existence of a major transcriptional activation of the heat-shock genes was never in doubt in the first place, because the specific puffs that are induced by heat shock are considered to be cytological indicators of transcriptional intensity. However, the molecular basis of polytene chromosome puffing is not well understood (24). RNA synthesis can be intense without puff formation (27), and therefore, it is probable that both the rate of transcription and the kinetics of hnRNA processing contribute to puff size (4). The possibility exists that puff formation is largely a posttranscriptional event in which hnRNPs particles accumulate near their sites of assembly (2). This same point has been raised by Bonner and Pardue (4).

**Prevalence of Drosophila Nuclear RNAs**

Some interesting points regarding nuclear transcript abundance emerge from the present study. As a frame of reference, we note that in fully induced Friend erythroleukemia cells, which can be considered to be a "rich" source of globin gene transcripts, 0.013% of the nuclear RNA labeled in a 5-min

\[^3H\]uridine pulse is β-globin specific (T. Pederson, unpublished data). This would typically be regarded as a prevalent nuclear RNA. Considered in this light, the transcriptional intensity of the Drosophila heat-shock genes is remarkable: ~1% of the nuclear RNA labeled in a 5-min pulse immediately after heat shock is 70,000-dalton heat-shock gene transcript, and this value increases yet further to ~2.5% of the nuclear RNA after a 30-min pulse (Table I). Thus, these heat-shock gene transcripts are two orders of magnitude more prevalent than are globin gene transcripts in a differentiated erythroid cell and, therefore, could reasonably be termed "superprevalent."

A second point concerns the transcription of actin and heat-shock protein genes at 25°C. Actin gene transcripts represent ~0.1% of the nuclear RNA labeled in a 5-min pulse, and transcripts of the 70,000-dalton heat-shock genes represent 0.045% of the nuclear RNA labeled in a 5-min pulse in 25°C cells (Table II). Thus, both actin and heat-shock gene transcripts in 25°C Drosophila cultured cells are in the same general range of prevalence as β-globin gene transcripts in induced Friend erythroleukemia cells (0.013%). Therefore, the heat-shock response would appear to represent a shift of one set of transcripts from a prevalent to superprevalent range (the 70,000-dalton heat-shock protein genes), and another (actin) from a prevalent to an almost undetectable level. These shifts are largely reversed to the original transcription patterns after heat shock (Figs. 2–4), showing that the levels of these transcripts are tightly coregulated.

**Heat Shock and the Cytoskeleton**

Although total RNA synthesis declines during heat shock, actin gene transcription is especially sensitive and is almost completely suppressed (Fig. 2). Return of heat-shocked cells to 25°C elicits a reactivation of actin gene transcription (Fig. 2). Although it is not presently clear why actin gene transcription should be so responsive to heat shock, there is evidence in cultured mammalian cells that the synthesis of cytoskeleton elements (including actin) is very sensitive to environmental signals, particularly ones relating to cell anchorage (9). The newly realized ability to turn Drosophila actin genes on and off (Fig. 2), in conjunction with the use of cloned Drosophila genes for other structural proteins such as tubulin, might now make it possible to exploit heat shock to dissect transcriptional and posttranscriptional controls that underly the biosynthesis and assembly of the cytoskeleton.

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