
***Copia* is transcriptionally responsive to environmental stress**

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ABSTRACT

Adult *Drosophila* subjected to a variety of environmental stresses that induce classic *Drosophila* heat shock response simultaneously exhibit a rapid and significant rise in *copia* homologous transcripts. Levels of *Drosophila Adh* (alcohol dehydrogenase gene) and 18s ribosomal RNA were unaffected by environmental stress. *Copia*'s ability to be induced by stress is correlated with the presence of sequences homologous to the heat shock promoter consensus sequence which appear to be appropriately positioned within the element's long terminal repeat (LTR). Although the *copia*-like element 297 also contains homologous sequences within its LTR, they are atypically positioned relative to the element's transcription start site and are non-functional in that the 297 element was not stress inducible. Hence, the position of the consensus sequence relative to a gene's transcription start site may be a factor in stress inducibility.

INTRODUCTION

Copia is a 5 kb retroviral-like movable genetic element in *Drosophila melanogaster* present in about 50 randomly dispersed copies per genome (1). *Copia* and *copia*-like families of genetic elements are believed to constitute at least 5% of a fly's total DNA. *Copia*-like elements are particularly interesting in that they display a remarkable similarity to the integrated form of mammalian RNA tumor viruses. For example, *copia*-like elements are bounded by long terminal direct repeats of 200-600 bp (2); closed circular extra-chromosomal *copia* DNA has been isolated from *Drosophila* culture cells (3); viral-like particles isolated from *Drosophila* culture cells have been found to contain high molecular weight RNA homologous to *copia* and proteins with reverse transcriptase activity (4). This latter finding is consistent with the fact that one of three open reading frames of the *copia*-like element 17.6 exhibits homology to genes encoding reverse transcriptases(5). Moreover, *copia*-like elements, like their retroviral analogues, are capable of transposition and a number of spontaneous and naturally occurring muta-

tions in Drosophila are the result of copia element insertions (1,6).

We report here that the copia element is responsive to a variety of environmental stresses that are known to activate the transcription of genes for heat shock proteins in Drosophila (7). In addition, we have correlated the shared responses of copia and the Drosophila heat shock genes with the presence of 14 bp regulatory sequences ("heat shock promoters") (8) located within 200 bp 5' to the respective genes' transcriptional start sites.

MATERIALS AND METHODS

Drosophila strains.

Drosophila melanogaster flies used in this study were derived from natural populations (9) and made completely homozygous according to previously published techniques (10). All strains were reared and maintained at 20°C prior to stress.

Stress treatments.

For temperature stress, approximately 50 adult flies (6-10 days post-eclosion) were placed in glass vials and subjected to 37°C for up to 5.0 hours. For chemical stress, flies were exposed for 1 hr to 1 ml of 30% hydrogen peroxide or 0.6 mM sodium azide solution absorbed onto filter paper (2.5 x 2.5 cm). Control flies were subjected to an equal volume of water. All experimental treatments were predetermined to be the maximum sub-lethal conditions for the exposure periods involved, and in no instance were flies killed by the stress treatments.

Molecular techniques.

Total RNA and polyadenylated RNA collected by oligo-dT chromatography (11,12) were fractionated on formaldehyde/agarose gels (12), transferred to nitrocellulose (13) and hybridized (14) to the following ³²P labeled (15) probes: cDm 5002 (Drosophila copia element) (16) cDm 4006 (Drosophila copia-like element 297) (16), p 229.1 (Drosophila heat shock protein 70, hsp-70) (17), psAC1 (Drosophila alcohol dehydrogenase gene, Adh) (18), pSr 1.235 (18S ribosomal RNA gene) (19).

Gels were exposed to Kodak XAR-5 film for varying periods of time and the resulting autoradiograms scanned at 550 nm in a Beckman Du-8 spectrophotometer equipped with a program to compute areas under peaks of optical density.

RESULTS

Temperature stress

The results of Northern analyses using total Drosophila RNA (Fig 1) demonstrate a rapid and significant rise in levels of copia (1a) and hsp-70 (1c) homologous transcripts in response to heat stress. Hybridization of Drosophila Adh (sAC1) and 18S ribosomal RNA (pSr1.235) probes to these same filters (1b,e) demonstrated no differential response between stressed and non-stressed flies at these loci. Thus, the heat shock response of copia is not an artifact of unequal sample loading nor is it a response which is shared by all Drosophila genes. The response was maximal within 30 minutes of heat treatment. As can be seen in the Table, the level of induction of the 5kb copia transcript at 30 min averaged >6-fold (range: 3-fold to 13-fold). Transcripts of the copia type element 297 were not stress inducible above the trace levels present in unstressed adults (average fold induction 1.4 ± 0.5).

Chemical stress

Besides heat, Drosophila heat shock genes transcriptionally respond to a variety of chemical agents (7) and the same apparently holds for copia. Figure 1d illustrates copia's response to hydrogen peroxide and sodium azide both of which induce the heat shock response in Drosophila (20,21).

Figure 2 displays an autoradiograph of polyadenylated RNA from H_2O_2 stressed flies which has been hybridized to labeled copia DNA. A 2 kb copia transcript not apparent in autoradiographs of total RNA (fig 1d), is clearly present here. It has previously been reported that transfer of the 2 kb copia transcript is blocked by an abundant class of ribosomal RNA in Northern of total RNA (22). In polyadenylated RNA isolated from stressed flies, there may be another copia homologous transcript of ~ 3 kb in length (Fig 2). Although we have observed this band in other experiments, the putative transcript is wide and poorly resolved above background. Thus further analysis is required before any definitive conclusions can be made.

Sequence search

Since copia appears to display a stress response similar to the Drosophila heat shock genes, we conducted a sequence search of its LTR for possible homology with the promoter regions of the heat shock genes. Deletion analysis of the 5' flanking region of the hsp-70 gene have identified a sequence between positions -47 and -66 from transcription start site necessary for the heat shock response (8). This region contains a 14 bp

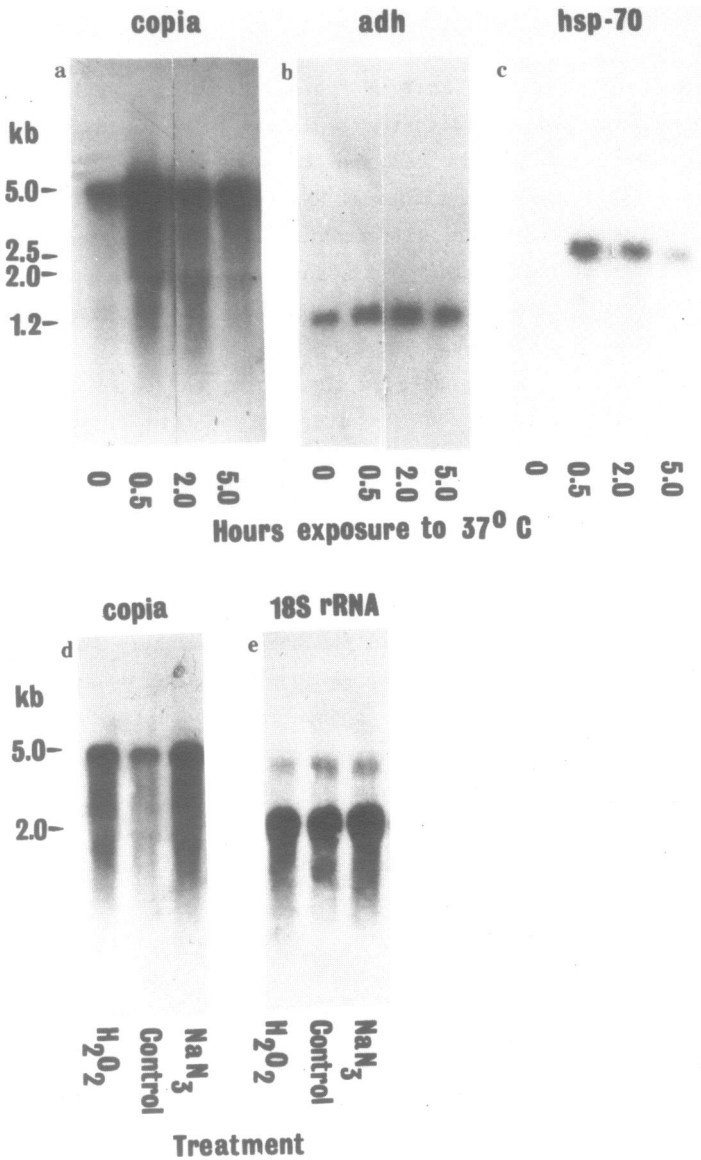


FIGURE 1. Northern blots of total *Drosophila* RNA demonstrating the effect of environmental stress on copia (a,d), Adh (b), hsp-70 (c) and 18S rRNA (e) transcript levels in adult *Drosophila melanogaster*. Identical amounts of total RNA sample was loaded in each lane. Radioactive signal was allowed to decay on test filters (a,d) prior to rehybridization to control probes (b,c,e). (Relative level of copia induction over the time course of this experiment: 0 hrs.=1.00; 0.5 hrs.=3.50; 1.0 hrs.=2.50; 2.0 hrs.=1.30; 5 hrs.=1.20, as determined by densitometric scans of autoradiographs.)

Table 1. Adh and copia homologous transcript levels* present in control (22°C) and stressed (37°C) adult Drosophila.

Experiment	CONTROL		STRESSED		INDUCTION RATIO**	
	<u>Adh</u>	<u>copia</u>	<u>Adh</u>	<u>copia</u>	<u>Adh</u>	<u>copia</u>
1	1.0	4.4	1.6	22.8	1.6	5.2
2	2.4	0.3	3.7	4.2	1.5	12.7
3	1.0	0.5	0.8	1.3	0.8	2.6
4	11.0	0.5	17.0	3.4	1.5	6.2
5	2.4	2.2	2.2	10.0	0.9	4.5
Average (S.E)					1.3 (0.2)	6.2 (1.7)

* values are areas under absorbance peaks (cm^2).

** ratio of stress: control values

sequence beginning at -49 which is homologous to the promoter regions of all of the heat-shock genes of Drosophila (Figure 3). These experiments were carried out by transformation of COS cells or microinjection of DNA into Xenopus oocytes (23). However, Dudler and Travers have transformed Drosophila with hsp-70 deletions and found that sequences up to position 68 from the gene's transcription start site affect the hsp-70 gene's heat shock response (24). They also noted that the region -68 to -97 of the hsp-70 gene contains a second heat shock promoter consensus sequence (24).

By computer searching, we have also found (Figure 3) two or more copies of the heat shock consensus sequence within 200 bp of the 5' end of the respective initiation sites of all the Drosophila heat shock genes (Fig. 3). Whether or not the presence of several consensus sequences is prerequisite for normal heat shock response is unknown. It is worth noting that the Drosophila heat-shock consensus sequence is part of an inverted repeat which

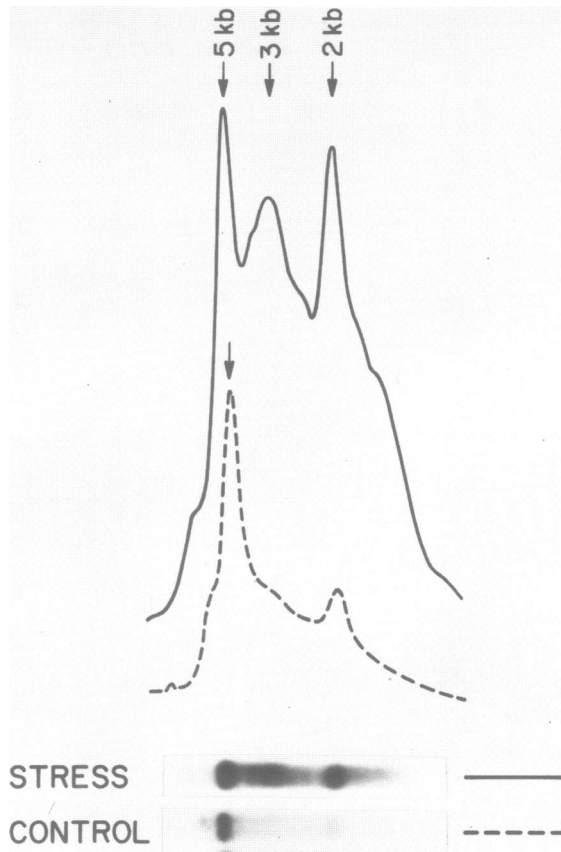


FIGURE 2. Densitometric scan of polyadenylated RNA isolated from control and hydrogen peroxide stressed adult *Drosophila*. Identical amounts of control and test polyadenylated RNA sample were loaded in each lane. Intensities quantitated by densitometric scans of autoradiographs.

has recently been shown to bind an RNA polymerase II transcription factor required for heat induced transcription at the *hsp-70* locus (25,26).

Figure 3 summarizes homologies among the *Drosophila* heat-shock promoter sequences and the LTR regions of *copia* and *297*. Homology was calculated in two ways. In one, the percent homology is computed between a given sequence and the entire 14 bp heat shock consensus. Within this 14 bp sequence is a 10 bp palindrome which has been proposed by Pelham and Bienz as the essential component of the regulatory element (23). Thus, we also computed the percent homology between the heat shock genes and this 10 bp sequence. What emerges from these comparisons is that *copia* contains a 14 bp sequence in

GENE	HEAT SHOCK SEQUENCE	BP FROM TRANSCRIPTIONAL START	% HOMOMOLOGY WITH CONSENSUS
H.S. consensus	<u>CTgGAAtnTTcTAg</u>		100
<u>hsp-22</u>	<u>CCGGTATTTTCTAG</u>	-63	86(80)
	<u>GAAGAAAATTCGAG</u>	-83	64(80)
	<u>CAGAAACTTTCACG</u>	-183	64(70)
<u>hsp-23</u>	<u>GTCGATGTTTGTGC</u>	-108	50(50)
	<u>CGTGTCCCTTCTCG</u>	-124	50(60)
	<u>CGAGAAGTTTCGTG</u>	-134	64(80)
	<u>GCGCAAATTCGAG</u>	-169	64(70)
<u>hsp-26</u>	<u>CCGGACTCTTCTAG</u>	-50	86(80)
	<u>CTCTACTCTTTCCT</u>	-78	50(50)
	<u>AAGCTATATTCATG</u>	-134	57(50)
<u>hsp-27</u>	<u>TTGCCATGCACTAG</u>	-55	64(50)
	<u>ATTAAAAGTTCGGTC</u>	-74	43(50)
	<u>GGAAAACCTTCTGC</u>	-127	50(50)
	<u>GGGGCGTATTCCAA</u>	-236	57(50)
<u>hsp-68</u>	<u>CTCGAATTTTCCCC</u>	-49	71(80)
	<u>CTGGAATGTTCTGA</u>	-82	86(80)
<u>hsp-70</u>	<u>TGCGAATGTTCCGG</u>	-50	64(70)
	<u>CTCGTTGCTTCGAG</u>	-75	64(80)
<u>hsp-83</u>	<u>CTAGAAGTTTCTAG</u>	-62	86(100)
	<u>CCAGAAGCCTCTAG</u>	-72	71(80)
<u>copia</u>	<u>CTACAAAATAACG</u>	-90	50(60)
	<u>TTGGAATATACTAT</u>	-110	86(70)
<u>297</u>	<u>TAGTAATTTTCCAT</u>	-204	64(60)
	<u>GTGGACCAACCAG</u>	-265	57(60)
	<u>CTTTCACCGTCCAG</u>	-364	57(70)

FIGURE 3. Homologies between the *Drosophila* heat shock promoters and LTR sequences of the *Drosophila* movable elements *copia* and *297*. The sequences were aligned with respect to the *Drosophila* heat shock consensus promoter utilizing a computerized sequence search. Underscoring indicates a match with the 14 bp consensus. Percent homology was calculated in two ways as indicated in the text. The first value is the homology which exists between the indicated sequence and the entire 14 bp consensus. The second value (in brackets) is the homology which exists between the indicated sequence and the 10bp palindrome contained within the 14 bp consensus (uppercase). The sequences are from Flavell et. al. (38), *copia*; Ikenaga and Saigo (39), *297*; Ingolia and Craig (40), *hsp-23*; Holmgren et. al. (41), *hsp-68*; and Torok and Karch (42), *hsp-70*.

its LTR which is homologous to the heat shock consensus.

Copia's putative stress control sequences are positioned -90 and -110 bp, respectively, from the element's transcription start site. In contrast, homologous sequences contained within the LTR of copia-like element 297, which is not stress inducible, are further upstream (-204, -267 and -364) and further apart from each other than in copia or any of the Drosophila heat shock genes. Whether or not this distant positioning of the consensus homologous sequences within the element 297 is responsible for lack of response to heat shock has yet to be determined.

DISCUSSION

We cannot, at present, formally exclude the possibility that the stress-induced increase in copia transcript levels is totally due to post-transcriptional stabilization. We believe, however, that there are compelling reasons for concluding otherwise. Stress-induced transcription of Drosophila genes has previously been associated with a 14 bp sequence (23,24,25) that we find present and appropriately positioned within copia's LTR. In addition, we have recently demonstrated that a copia LTR fused to the bacterial chloramphenicol transferase gene (CAT) (27) is transcriptionally activated when transfected into rat cells subjected to mammalian heat shock conditions (28).

The potential significance of transcriptional activation of copia-like elements is twofold. First, recent evidence indicates that retroviral-like mobile elements replicate by reverse transcription of RNA intermediates (29). This suggests that stress-induced transcription of copia-like elements may induce an increase in insertional mutation rates as well. Although unambiguous evidence has yet to be acquired in support of this hypothesis, it is consistent with previous observations that temperature and other genomic stresses are correlated with increased mobile element transposition rates in maize (30), yeast (31,32) and Drosophila (33).

Second, stress-induced transcription of copia-like elements may have potential regulatory effects on adjacent sequences. Copia-like elements can be viewed as "mobile promoter sequences", i.e., their insertion into a gene's regulatory region may place adjacent sequences under novel promotional control (see references 34, 35). In this regard, our results raise the possibility that appropriately positioned copia insertions may render adjacent sequences "stress-inducible". Since it appears that an LTR remains when copia elements excise (36,37) it is interesting to speculate that the

element may have contributed to the dispersal of stress-inducible promoter sequences over evolutionary time.

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