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Transcription of *Drosophila* mobile element *gypsy* (mdg4) in heat-shocked cells

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Drosophila melanogaster Schneider 2 line cultured cells were subjected to stable transformation by co-transfection with two plasmids, one of which conferred G418 resistance and another which contained the *Drosophila* retrotransposon, *gypsy* (mdg4), under the control of the heat-shock protein 70 promoter. Transcription of the introduced constructs, as well as of endogenous *gypsy*, was examined under the condition of heat shock. Active degradation of pre-existing *gypsy* transcripts was observed. During recovery, *gypsy* transcription was restored, but its termination and/or 3′-end processing became aberrant.

Retrotransposon *gypsy*; *Drosophila melanogaster* cultured cell; Transfection; Heat shock; Transcription

1. INTRODUCTION

Retrotransposons are the representatives of a wide class of *Drosophila* mobile genetic elements, which are considered to be important factors of genetic instability [1]. Mechanisms of the regulation of their expression are rather complicated and are not yet well understood, so it was of interest to create a model system in which retrotransposons were under the control of an inducible promoter. For this purpose, we chose *gypsy* (mdg4); its nucleotide sequence and some details of its transcription regulation has been determined [2–4]. Plasmids containing *gypsy* under the control of the promoter of the *Drosophila* heat-shock protein 70 (hsp70) gene [5] were constructed, and heat shock was used to induce *gypsy* expression. DNA constructs were introduced into *Drosophila* cultured cells and *gypsy* transcription was examined under the condition of heat shock.

2. MATERIALS AND METHODS

2.1. *Plasmid constructions*

To construct phs1*GYP*, 0.4 kb *Xho*I–*Sau*3A fragment from pUChsneo [6], containing the promoter and leader regions of the hsp70 gene, was cloned in pUC19 into *Sal*GI and *Bam*HI sites. Then the 1.3 kb *Sau*3A–*Pst*I and 4.3 kb *Pst*I–*Eco*RI *gypsy* fragments from plasmid Dm111 [7] were introduced into *Bam*HI and *Eco*RI sites of the previously obtained plasmid, and finally a 2.2 kb *Eco*RI fragment from Dm111 was cloned into the *Eco*RI site. Selection of clones containing this fragment in the proper orientation was then performed.

To construct pHS*GYP*, the *Bam*HI–*Pst*I fragment from Dm111 was cloned into pUC19, then the 0.45 kb *Bam*HI–*Xho*I fragment was substituted by the 0.45 kb *Bam*HI–*Sal*GI fragment from the hsp70 promoter [5], and finally the corresponding *Pst*I fragment from Dm111 was inserted in the proper orientation.

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2.2. *Preparation and treatment of nucleic acids*

Plasmid DNA extraction, restriction enzyme treatment, DNA labelling, poly(A)$^+$ RNA isolation and Northern blotting experiments were performed according to Maniatis et al. [8].

2.3. *Transfection*

gypsy constructs were introduced into *D melanogaster* Schneider 2 cells by co-transfection with pUChsneo [6], containing G418 resistance, by a standard calcium phosphate procedure according to DiNocera and Dawid [9]. G418 was added 48 h after the transfection and, after 15–20 days, separate G418-resistant clones were obtained and examined for *gypsy* construct expression.

2.4. *Heat shock and recovery*

Heat shock and recovery were performed at 37°C and 25°C, respectively, for different periods of time, and after that RNA was quickly isolated. All the procedures of RNA extraction were performed at 0°C.

3. RESULTS AND DISCUSSION

Fig. 1 shows the restriction maps of the *gypsy* constructs used. phs1*GYP* (Fig. 1) contains the hsp70 gene promoter and the non-translated leader region (from −180 to +206 according to the transcription initiation start site) [6], and the *gypsy* fragment, including a small part of the leader region, all coding sequences and right LTR (missing its own promoter) and regions involved in its transcription regulation. Therefore, translation as well as transcription of this construct should be controlled by heat shock. pHS*GYP* (Fig. 1) contains only the hsp70 gene promoter (from −180 to −30) and practically the entire *gypsy* sequence, including the transcription initiation start site as well as positive and neg-

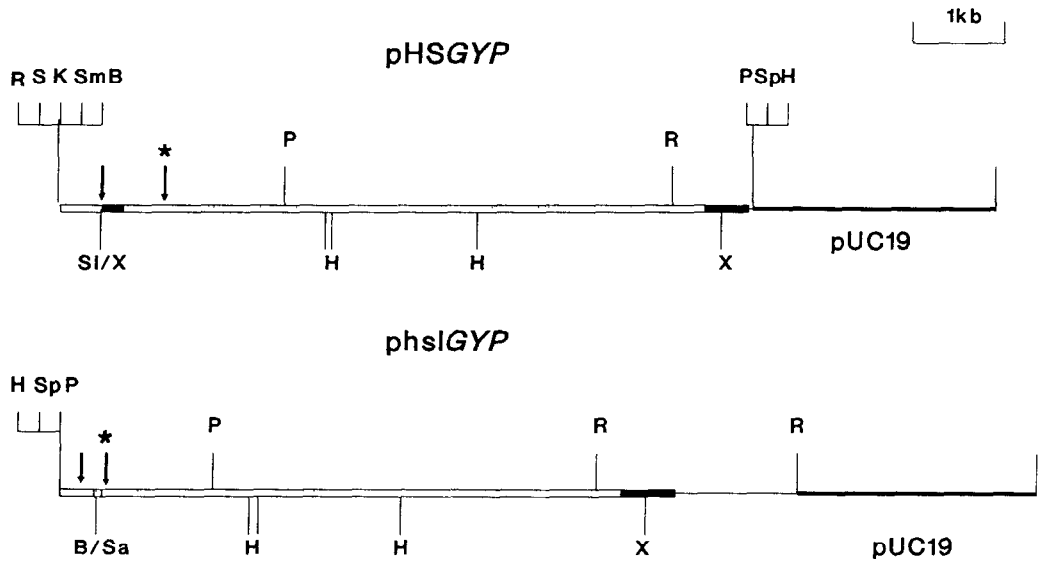

Fig 1. Restriction maps of pHS*GYP* and phs1*GYP* constructs. Restriction enzyme abbreviations are. R, *Eco*RI; S, *Sst*I; K, *Kpn*I; Sm, *Sma*I; B, *Bam*HI; Sl, *Sal*GI; X, *Xho*I; P, *Pst*I; H, *Hin*dIII; Sp, *Sph*I. Open, filled and dotted boxes confine hsp70 promoter sequences, *gypsy* LTRs and internal sequences, respectively Arrows indicate transcription start sites and arrows with asterisks indicate the first ATG codons.

ative regulatory regions [3,4] (from −35 to the end of element). In this case only the level of transcription should be controlled by heat shock, and the structure of the transcript and its translation should be regulated according to the *gypsy* sequences.

Even preliminary experiments on Northern blot analysis of RNA isolated from transformed *Drosophila* cells showed the non-trivial character of endogenous *gypsy* transcription under conditions of heat shock. That is why the transcription of this element was also investigated in non-transformed Schneider 2 cells under the same conditions.

Fig. 2 presents the results of Northern blot analysis of poly(A)$^+$ RNA isolated from Schneider 2 cells trans-

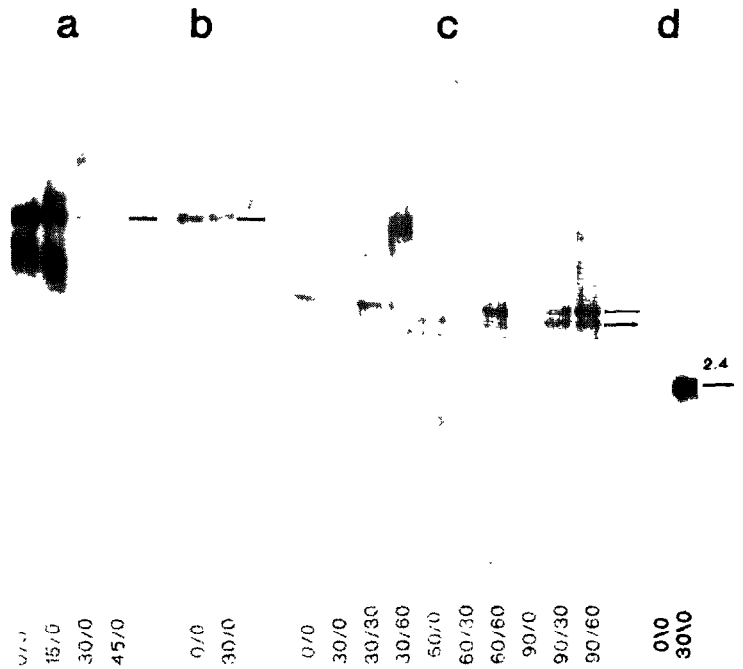

Fig. 2. Northern blot analysis of *gypsy* poly(A)$^+$ RNA extracted from Schneider 2 cultured cells, non-transformed (a) and transformed by pHS*GYP* (b) and phs1*GYP* (c) constructs Numbers below indicate time of heat shock/recovery in min.

formed by *gypsy* constructs (b,c), as well as non-transformed cells (a), under different conditions of heat shock (time of heat shock and recovery varied). These data show not only the interruption of *gypsy* transcription in heat-shocked cells, but also the degradation of pre-existing transcripts since during 45 min of heat shock they are completely eliminated. In addition to the elimination of the 7 kb endogenous *gypsy* transcripts, phs1*GYP*-transformed cells exhibit the appearance of 6.5 kb transcripts corresponding to the phs1*GYP* construct (Fig. 2c). During recovery from the heat shock, transcription of the introduced phs1*GYP* construct ceases and induced transcripts gradually degrade, while transcription of endogenous *gypsy* increases. The experiments on verification of the duration of heat shock and following recovery demonstrate that longer heat-shock treatment needs more recovery time to restore *gypsy* transcription back to initial levels. In pHS*GYP*-transformed cells (Fig. 2b) the pattern of *gypsy* transcription during heat shock seems not to change, but that is probably the result of two processes, (i) the degradation of previously synthesized *gypsy* transcripts, and (ii) the appearance of heat shock-induced pHS*GYP* transcripts which have the same size.

Monitoring of RNA isolation and heat-shock effect was performed by rehybridization of the same filters with actin and hsp70 genes (as an example, Fig. 2d demonstrates the rehybridization of the filter shown in Fig. 2b to hsp70 DNA).

It is well known that heat shock causes an interruption of synthesis of almost all RNAs except those coding for so-called heat-shock proteins [10–12]. In this connection it seems interesting that in this work we demonstrated not only the interruption of transcription of one of *Drosophila*'s retrotransposons but also the active degradation of its pre-existing transcripts, since during 45 min of heat shock endogenous *gypsy* transcripts are eliminated (the determined half-life of *gypsy* RNA is about 2 h). Fig. 3 presents the Northern blot analysis of transcription of two other *D. melanogaster* retrotransposons, *copia* [13] and mdg1 [14], in heat-shocked Schneider 2 cells, as well as *gypsy* transcription in two other *D. melanogaster* cell lines. These data demonstrate that active degradation of pre-existing transcripts is observed only for *gypsy*, and not for *copia* and mdg1 (compare Fig. 2a and Fig. 3a,b), and only in Schneider 2 cells, but not in 67J25D [5] (Fig. 3c) or Kc cells [16] (Fig. 3e). In 67J25D cells heat shock affects neither the stability of the main transcript nor any of the other additional transcripts which are usually observed in this cell line, and may represent transcription of some defective *gypsy* copies.

There are some chemical agents (e.g. peroxide, ethanol) which cause the same effect as heat shock [10–12], however, in this case these agents did not cause specific degradation of *gypsy* transcripts in Schneider 2 cells (data not shown). All these experimental data suggest the existence of protein factor(s) responsible for the stability of *gypsy* RNAs which are thermolabile in Schneider 2 cells.

It is known that heat shock may influence not only transcription but also post-transcriptional RNA processing (e.g. splicing, termination) [17,18]. The analysis of data presented in Fig. 4 and obtained from the exper-

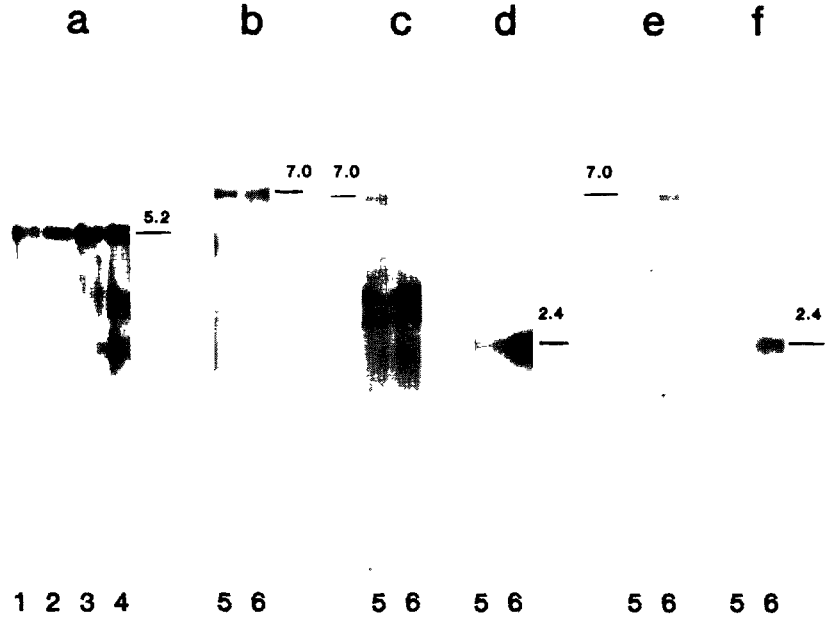

Fig. 3. Northern blot analysis of *copia* (a), mdg1 (b), *gypsy* (c,e), and hsp70 (d,f) poly(A)$^+$ RNA isolated from Schneider 2 (a,b), 67J25D (c,d) and Kc (e,f) cultured cells which were not heat shocked (lanes 1,5) or incubated at 37°C for 15 (lane 2), 30 (lanes 3,6) and 45 (lane 4) min.

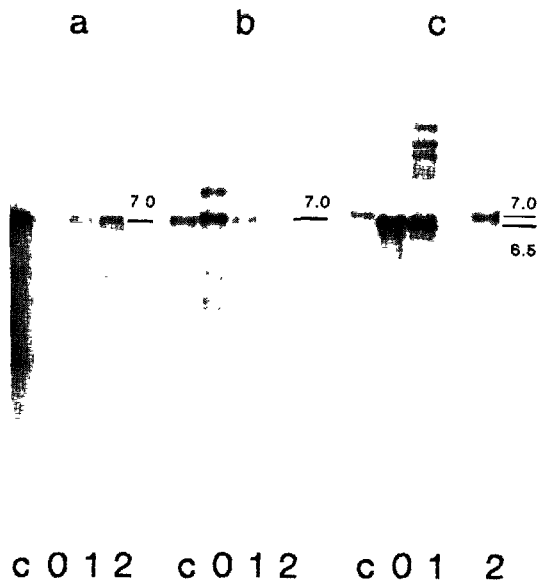

Fig. 4. Northern blot analysis of *gypsy* poly(A)$^+$ RNA isolated from non-transformed cells (a) and cells transformed with pHS*GYP* (b) and phs1*GYP* (c) which were not heat shocked (lanes c) or incubated at 37°C for 30 min with further recovery for 0, 1 or 2 h

iments when cells were incubated at 25°C after heat shock for different periods of time, shows that during recovery *gypsy* transcription is restored, but initially its termination and/or polyadenylation of endogenous *gypsy* RNAs occur not in right LTR (long terminal repeat) but elsewhere in adjacent downstream sequences, leading to the appearance of discrete patterns of corresponding polyadenylated transcripts of larger size. It is interesting that poly(A)$^+$ RNAs larger than 7 kb are observed during restoration of *gypsy* transcription in non-transformed cells (Fig. 4a, lane 1), as well as during heat shock-induced expression of phs1*GYP* and pHS*GYP* constructs (Fig. 4b, lane 0 and Fig. 4c, lane 0). During further recovery larger transcripts disappear and the synthesis of normal transcripts is restored back to the initial level (Fig. 4b,c, lanes 2).

It should be mentioned that in the case of phs1*GYP* construct violation of transcription termination is not as well expressed as for the pHS*GYP* construct, since the intensity of the RNA band corresponding to the normal transcripts is higher in the first case. This fact may be explained by the presence of the non-translated hsp70 leader region, as well as by the absence of *gypsy* sequences responsible for transcription regulation in phs1*GYP* [3,4]. It is not clear, however, how leader sequences may influence the processes of transcription termination and 3′-end processing. Since phs1*GYP* also contains the 1.5 kb fragment from Dm111 adjacent to the 3′ LTR it cannot be excluded that this sequence may be responsible for more accurate termination in phs1*GYP*.

It is also worth mentioning that the level of RNA synthesis induced by heat shock was less than expected from the literature [6,19]. The existence of some mechanisms regulating the rate of *gypsy* RNA accumulation in *D. melanogaster* cells seems likely.

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