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The retrotransposon *copia* regulates *Drosophila* gene expression both positively and negatively

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ABSTRACT

The *D. melanogaster* transposable element *copia* is structurally similar to retroviral proviruses. We have asked whether *copia* encodes regulatory functions which have been observed in certain other proviruses. We have introduced reporter constructs based on the *copia* promoter and other *Drosophila* promoters into *Drosophila* cells and asked if *copia* has any affect on their expression. We find that, whilst *copia* negatively regulates expression from its own promoter, it also positively regulates expression from the larval serum protein 1 promoter. Analysis of RNA suggests that both regulatory functions occur by post-transcriptional mechanisms.

INTRODUCTION

The *Drosophila* transposable element (TE) *copia* is a retrotransposon which is structurally similar to retroviral proviruses and is present in cultured *D. melanogaster* cells at approximately 100 copies/cell (1). The element is 5kb in length, flanked by 276 bp direct long terminal repeats (LTRs) and the nucleotide sequence shows a single large open reading frame (ORF) capable of encoding a polyprotein of 1409 amino acids (2,3). By sequence analysis, the polyprotein is believed to encode products similar to those encoded by other retroviruses which include a putative nucleic acid binding protein, a protease, integrase and a reverse transcriptase (2,3). However, these products have not been demonstrated directly.

In cultured cells, two major transcripts of 5 kb and 2 kb are observed. These represent a full transcript beginning in the 5′ LTR and terminating in the 3′ LTR (4) and a spliced 2kb product with identical 5′ and 3′ ends but lacking a 3kb sequence which encodes the putative *int* and *pol copia* gene products (5). In cultured cells, which contain high levels of VLPs and other transposition intermediates (6), the 5kb and 2kb RNAs are equimolar. Whereas in flies, which contain few virus like particles (7), the 5kb RNA is far more abundant than the 2kb species. This is consistent with the 2kb RNA being able to direct the synthesis of *copia* virus like particles (VLPs) in cultured cells (7).

Over the last few years it has been shown that some retroviruses encode regulatory functions which are believed to both positively and negatively regulate viral (8) and cellular (9) gene expression. We have asked whether the *copia* element also encodes regulatory functions by analysis of the effect of *copia* on its own expression and that of other *Drosophila* genes. Here we show that the *copia* element negatively regulates expression from its own LTR and positively regulates expression of at least one *Drosophila* cellular gene promoter. Lastly, we show that this transregulation function is encoded by the 2kb *copia* RNA product and is likely to act post-transcriptionally.

MATERIALS AND METHODS

Plasmids

*copiacat*1 (10), LSP1*cat* (11) pIEP1*cat* (12), and r*cat*5.3 (13) are CAT expression vectors under the control of a single *copia* LTR, the *Drosophila* LSP1a promoter, the human cytomegalovirus major immediate early promoter and the *Drosophila rudimentary* gene respectively. These constructs are detailed in figure 1. p11.4 is a full genomic *copia* element (14) and deletion derivatives were constructed by restriction enzyme digestion, blunt ending with mung bean nuclease and religation. pCB13′LTR is a *copia* LTR driven expression vector for the 2kb *copia*-specific cDNA (15). pDV111 is a *Drosophila* genomic rDNA probe (16). HS*cat* was constructed by replacing the *Hind*III/*Bam*H1 *gpt* fragment of HS*gpt* (17) with a *Hind*III/*Bam*H1 *cat* fragment of SV2*cat* (18).

Cell culture and transfection

The *D. hydei* cell line, DH33 (19) and the *D. melanogaster* cell line, SL2 (20) have been described. Cells were maintained in M3 medium with 10% foetal calf serum (21) and transfected as described (22). Routinely 1μg of *105opiacat*1, 5μg of LSP1*cat*, 0.5μg of pIEP1*cat*, 0.1μg of HS*cat* or 0.1μg of r*cat*5.3 were co-transfected with 5–15μg of effector DNA (see figure legends). These levels of DNA were non-saturating with respect to CAT reporter expression and resulted in basal levels of CAT activity amenable to transactivation and transrepression assays. The amount of reporter construct transfected reflects the relative promoter strengths in the cell lines.

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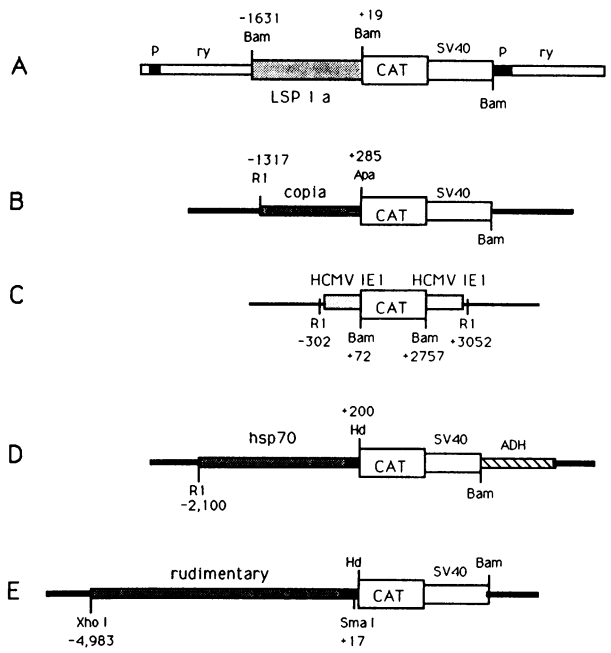

Figure 1. Expression vectors. (A) LSP1*cat*, (B) *copiacat*1, (C) pIEP1*cat*, (D) HS*cat*, and (E) r*cat*5.3. All vectors contain the chloramphenicol acetyl transferase gene (CAT) as well as the SV40 T intron splice and polyadenylation signals (SV40) from pSV2*cat* (18) except pIEP1*cat* which uses the HCMV IE1 polyadenylation signal. Promoter and flanking sequences are as indicated and details of all the constructions are published. Restriction site abbreviations are: Bam, *Bam* HI; R1, *Eco*RI; Apa, *Apa*I; Hd, *Hind*III. Other abbreviations: ry, *Drosophila rosy* coding sequences; SV40, SV40 polyadenylation and splice sequences; HCMV IE1, the human cytomegalovirus major immediate early gene; ADH, *Drosophila* alcohol dehydrogenase coding sequences; hsp70, *Drosophila* 70,000 mw heat shock protein coding region; p, plasmid sequences.

RNA analysis

Isolation (23) and analysis of steady state levels of RNA by slot-blots from co-transfected cells were carried out as previously described (24) except that RNA samples were digested with RNase-free DNase1 for 1 hour to remove residual transfected DNA. Slot-blots were probed with a CAT specific insert for 48 hours then stripped and reprobed with pDV111, a *Drosophila* rDNA genomic clone (16). Nuclear 'run on' assays were carried out as described previously (25).

CAT assays

CAT expression was assayed 96 hours post-transfection as described by Gorman *et al.* (18). The results shown are typical assays and have been reproduced in 5 independent experiments for *copiacat*1 and 5 independent experiments for LSP1a*cat*.

RESULTS

We have previously observed that expression of *copia*-based reporter constructs is higher in *D.hydei* cells, which do not contain endogenous *copia* elements, compared to *D. melanogaster* cell lines, which typically contain more than 100 copies per genome (17). This would be consistent with autoregulation of introduced *copia* LTRs by endogenous *copia* elements. However, alternative explanations could be that the *copia* LTR is more active in DH33 cells or factors necessary for *copia* LTR expression are limiting in *D. melanogaster* cells due to the high number of endogenous *copia* elements.

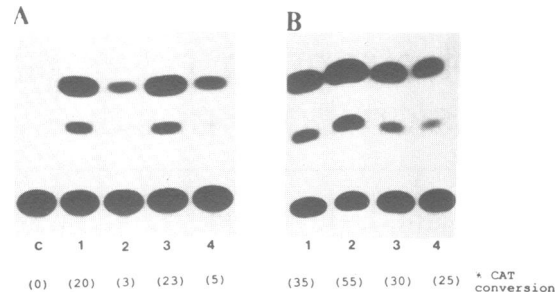

Figure 2. *copia* negatively autoregulates. DH33 cells were co-transfected with 1ug of *copiacat*1 (A) or 0.5 μg of pIEP1*cat* (B) together with 5 μg of either pUC19 (1), p11.4 (2), a *Stu*I/*Hpa*I deletion of p11.4 (3) or pCB13'LTR (4). C is control untransfected cells. % CAT conversions are shown in brackets.

To determine, directly, whether *copia* has an autoregulatory function a *copia*-CAT reporter gene, *copiacat*1, was co-introduced into *copia*-free DH33 cells with the *copia* containing p11.4. Fig. 2A clearly shows that the presence of p11.4 results in a major decrease in CAT expression from *copiacat*1. This repression is specific for the *copia* LTR as expression of pIEP1*cat*, the human cytomegalovirus (HCMV) major immediate early (IE) promoter *cat* construct which functions in *Drosophila* cells (12), is not altered by p11.4 (fig. 2B). This observation is not specific for *copia*-free *D. hydei* cells as similar results are seen if these co-transfections are carried out in *D. melanogaster* SL2 cells (data not shown).

We next carried out serial deletions of p11.4 to grossly define the region of *copia* responsible for this autoregulation phenomenon. Fig. 2A also shows that a minimal deletion from *Stu*I to *Hpa*I of p11.4 no longer represses expression of *copiacat*1 in DH33 cells. Similarly, deletions from *Apa*I, *Afl*II or *Nco*I to *Hpa*I (see Fig. 4) no longer repress expression of *copiacat*1 (data not shown).

As the splice acceptor site for the 2kb spliced *copia* product is at nucleotide 4551 just 5' to the *Hpa*I site (5), none of the p11.4 deletions would be likely to express the 2kb *copia* specific RNA (15). Consequently, we asked whether this spliced *copia* product was sufficient for autoregulation directly. Fig. 2A shows that pCB13'LTR, which is a cloned cDNA of the 2kb copia product under the control of a *copia* LTR (15), is sufficient to autoregulate *copiacat*1 expression in DH33 cells.

During this work, we also analysed the effect of p11.4 expression on other *Drosophila* promoters. Figure 3A shows the effect of p11.4 on expression of a *D. melanogaster* larval serum protein 1 (LSP1) promoter *cat* construct (11) in *D. melanogaster* SL2 cells. In contrast to the *copia* promoter, p11.4 strongly activates expression of the LSP1 promoter. Again, this activation is specific as the HCMV major IE promoter is not upregulated by *copia*. Similarly, figure 3C and 3D show that two other *Drosophila* promoters tested, the HSP70 promoter (which has relatively high basal activity in our hands) and the *rudimentary* promoter are not affected by p11.4. It was necessary to use SL2 cells in these experiments rather than DH33 cells because the LSP1*cat* and r*cat*5.3 vectors are not expressed to any detectable level in *D. hydei* cell lines (data not shown).

Similarly, co-transfection experiments with deletions of p11.4 and with pCB13'LTR clearly show that p11.4 sequences necessary for *copia* autoregulation are also responsible for the positive regulation of the LSP1 promoter (fig. 3A).

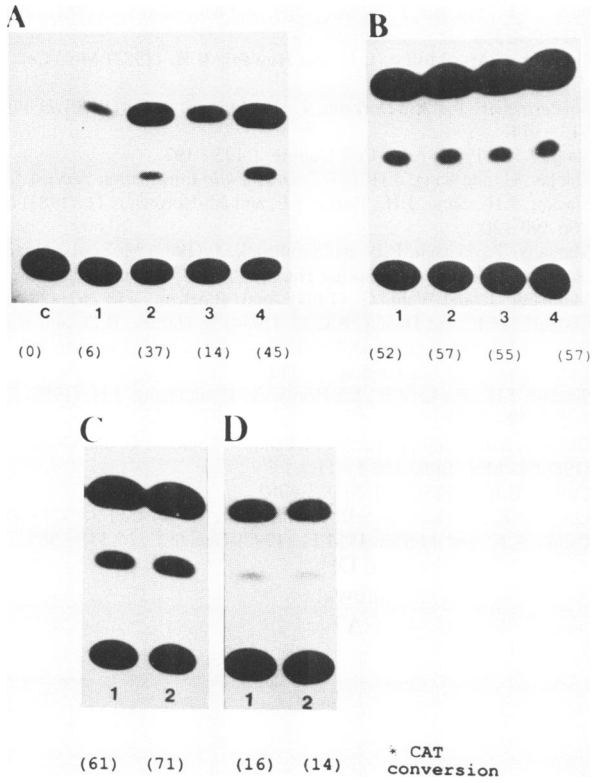

Figure 3. *copia* positively regulates the LSP1a promoter. SL2 cells were co-transfected with 5 μg of LSP1*cat* (A), 0.5 μg of pIEP1*cat* (B), 0.1 μg of pr*cat*5.3 (C) or 0.1 μg of pHS*cat* (D) together with 15 μg of either pUC19 (1), p11.4 (2), a *StuI/HpaI* deletion of p11.4 (3) or pCB13'LTR (4). C is control untransfected cells. % CAT conversions are shown in brackets.

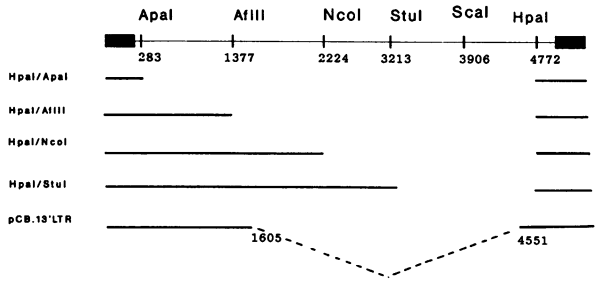

Figure 4. p11.4 deletions.

We have also analysed the positive and negative regulation of the LSP1 and *copia* promoters, respectively, at the level of RNA. Total RNA from DH33 cells co-transfected with *copiacat*1 and p11.4 or pUC19 was isolated and analysed by slot blot hybridisation using a *cat* specific probe then a genomic rDNA probe. Blots were also finally treated with sodium hydroxide and reprobed with *cat* specific insert to confirm that observed signals were due to RNA (data not shown). Figure 5A shows that, as with CAT activity, the level of CAT RNA decreased when cells were co-transfected with *copiacat*1 in the presence of p11.4. This is consistent with trans-repression occurring at the level of transcription. However, analysis of *de novo* RNA transcription by 'run on' assays (fig. 5B) shows no major changes in the transcription rate of *cat* RNA arguing for a post-transcriptional regulation of *copiacat*1 expression. In contrast, levels of CAT

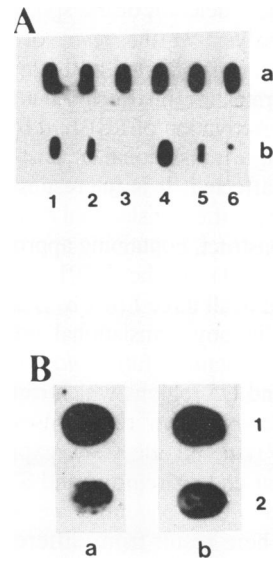

Figure 5. RNA analysis. A) Total cellular RNA was isolated from SL2 cells transfected with LSP1*cat* together with pUC19 (1) or p11.4 (2) or DH33 cells transfected with *copiacat*1 together with pUC19 (4) or p11.4 (5). RNA was slot-blotted and probed with a rDNA specific probe (a) then a *cat* specific probe (b). RNA from control untransfected SL2 (3) or DH33 (6) cells are also shown. B) Nuclei were isolated and labelled with [α-^{32}P]UTP from DH33 cells co-transfected with *copiacat*1 and pUC19 (a) or p11.4 (b). RNA was isolated and used to probe nitrocellulose filters that had been slot-blotted with 5 μg of linear pDV111 (1) or *cat* insert (2).

RNA did not increase when LSP1*cat* was co-transfected with p11.4 (see fig.5A), suggesting that transactivation of the LSP promoter by *copia* may occur at a translational or post-translational level.

Preliminary gel shift experiments using oligonucleotides specific for the *copia* LTR SV40-like enhancer (3) or AT rich motif (26) have so far shown no differences in nuclear factor binding between *copia*-free DH33 or *copia*-containing SL2 cells (data not shown). Whilst we cannot rule out technical explanations, this lack of difference in gel shifts would also be consistent with a post-transcriptional mechanism for autoregulation of the *copia* element.

DISCUSSION

We have shown that the *D. melanogaster* retrotransposon, *copia*, is not only capable of negatively regulating expression from its own promoter but also able to positively regulate expression from the *D. melanogaster* LSP1 promoter in transient DNA co-transfection assays. This transregulation is specific as other heterologous promoters such as the HCMV major IE promoter and Rous Sarcoma virus promoter (data not shown) or other homologous promoters such as the HSP70 or *rudimentary* promoters are not transregulated by *copia*.

By deletion analysis, both transregulatory functions map to the same region of *copia* in that deletion of sequences between *StuI* and *HpaI* result in p11.4 derivatives no longer capable of transregulation. Whilst these results would also be consistent with the 5kb RNA being responsible for transregulation, co-transfection of the spliced 2kb *copia* specific cDNA shows that this spliced product is, itself, sufficient for transregulation. Consistent with this is the observation that all deletions of p11.4 which do not

transregulate include a deletion of the splice acceptor site for the 2kb transcript. Analysis at the level of RNA suggests that repression of *copiacat*1 is effected at the level of RNA stability, since transcription rates are unaffected but steady state levels of RNA are lowered. Activation of LSP1*cat* by p11.4, on the other hand, must occur at a translational or post-translational level as RNA levels are unaffected. It is interesting to note that there is evidence for tissue specific translational control of LSP1 (27) and that the LSP1*cat* construct, containing approximately 200bp of 5′ untranslated leader (11) of the LSP1α gene, includes DNA sequences conserved in all three LSP1 α, β and genes (27) which could play a role in any translational control. Similarly, the *copiacat*1 construct contains a full *copia* LTR which includes the retroviral LTR R and U5 regions which can act as sites for post-transcriptional control in other retroviruses (28).

Except for pIEP1*cat*, all the CAT expression vectors used contain identical *cat* coding regions and SV40 polyadenylation and splice sequences. Consequently, we feel it is unlikely that the effects we see here result from differences in CAT coding sequences within the expression vectors.

Clearly, the ability of *copia* to regulate its own expression and regulate the expression of other cellular genes may have far reaching implications. Whilst *copia* induced mutations have been well documented (29), undefined *copia*-related long range effects on the expression of *Drosophila* genes have also been observed (30) and it is possible that transregulation by *copia* (whose own expression may be tissue specifically and developmentally regulated) may account for complex effects of TEs on the temporal and tissue specific expression of genes some distance from where they have integrated.

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