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Transformation of Cultured *Drosophila melanogaster* Cells with a Dominant Selectable Marker

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We have developed a method for the stable and efficient introduction of foreign DNA into *Drosophila melanogaster* tissue culture cells. A plasmid vector was constructed that carries the bacterial neomycin resistance gene under the transcriptional control of the copia transposable element long terminal repeat promoter. After calcium phosphate-DNA transfection, this vector rendered *D. melanogaster* cells resistant to the aminoglycoside G-418, a derivative of gentamicin. The vector DNA appeared to be integrated in long tandem arrays of 10 to 20 copies per cell and was stable for many generations in the absence of selection. To test the usefulness of this system for introducing nonselected DNA into *D. melanogaster* cells, a gene fusion between the P transposable element and the hsp70 promoter was inserted into the copia-neomycin resistance plasmid. After transfection and establishment of a G-418-resistant cell line, the hsp-P fusion gene was found to be efficiently transcribed after heat shock.

The introduction of cloned genes into recipient cells and organisms provides a powerful assay for the study of DNA sequences involved in transcription and translation and has made possible the expression of foreign gene products in eucaryotic cells. A variety of methods have been developed for the transformation of cultured eucaryotic cells with recombinant DNA. These include DNA transfection, microinjection, and protoplast or vesicle fusion (10, 16, 26, 35). The most widely used procedure involves treatment of cells with calcium phosphate-DNA coprecipitates (6, 51), which are internalized by phagocytosis. Transformed cells are then identified by their ability to survive under selective conditions that require expression of the exogenous DNA (4, 11, 23, 27, 37, 40, 50, 51). These cells usually contain multiple copies of the input DNA which may be integrated at one or more locations in the genome.

The fruit fly, *Drosophila melanogaster*, provides an excellent system for the study of many aspects of metazoan development. Classic genetic analysis has allowed the identification of many *D. melanogaster* genes whose products appear to be *trans*-acting regulators of gene expression (1, 19–22, 28, 43, 44, 48). However, a detailed understanding of the mode of action of these regulatory gene products will only be possible after their identification and biochemical characterization. The normal levels of these products might preclude their purification from various tissues or at different developmental stages of *D. melanogaster*. Therefore, it would be useful to develop a homologous system for the expression and overproduction of *D. melanogaster* gene products. A method for transforming *D. melanogaster* that uses the transposable P element as a vector for the introduction of foreign DNA sequences into the *D. melanogaster* germline has recently been developed (34, 41; see also references 15, 17, 36, and 42). With this method, it should be possible to devise vectors for the controlled high-level expression of rare gene products in the whole organism. Cell cultures, however, have two advantages for this application. First, high levels of many products which would be lethal to the organism may be tolerated by its cells in culture. Second, cell cultures provide a more uniform starting material for biochemical purification.

We have designed and tested plasmid vectors that allow the stable and efficient introduction of foreign DNA into *D. melanogaster* Schneider line 2 tissue culture cells. The vector, pcopneo, carries the bacterial neomycin resistance gene (*neo*), which confers resistance to the gentamicin derivative G-418 (11, 40), under the control of the promoter element in the long terminal repeat (LTR) of the copia transposable element. This vector was used to introduce a hybrid gene, in which P transposable element coding sequences are under the transcriptional control of the major heat shock protein (hsp70) promoter, into *D. melanogaster* cells. Our results indicate that this system allows the stable introduction of unselected DNA into *D. melanogaster* cells and that the expression of this newly introduced DNA is appropriately regulated.

MATERIALS AND METHODS

Recombinant DNA. The plasmid pcopneo was constructed by ligating the pAT153 vector (46) cleaved with *Bam*HI and *Eco*RI to a 2.5-kilobase (kb) *Hin*dIII-*Bam*HI fragment from plasmid pSV2-neo, which carries the neomycin resistance gene (40), and a 1.6-kb *Eco*RI-*Apa*I fragment carrying the copia transposable element LTR from plasmid cDm5002 (21). The *Apa*I terminus of the copia fragment was converted to an *Hin*dIII site by T4 DNA polymerase (P-L Biochemicals) treatment and ligation of synthetic 8-base-pair (bp) *Hin*dIII linkers (Collaborative Research).

Plasmid pNHP was constructed as follows. A 1.5-kb *Pst*I-*Sal*I fragment from plasmid pHT1 (31), carrying the hsp70 promoter, 1,100 bp of 5′ flanking DNA, and 90 bp of transcribed leader sequence, was subcloned into pUC8 (47), thereby placing a *Bam*HI restriction site 5′ to the hsp70 promoter sequences. This plasmid was then cleaved with *Eco*RI and *Hin*dIII, the sites for which are positioned 5′ and 3′, respectively, to the hsp70 insert. The ends of the resultant 1.5-kb *Eco*RI-*Hin*dIII fragment were made flush with Klenow fragment DNA polymerase I. In a separate set of reactions, the plasmid pπ25.7wc (18), which carries the complete P transposable element coding sequences, was partially digested with *Hin*dIII and recircularized. A plasmid

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from which the 1.1-kb *Hin*dIII fragment had been deleted was selected. In this plasmid, a portion of the P element promoter had been removed and the *Hin*dIII site at nucleotide (nt) 40 of the P element sequences (30) was now adjacent to the *Cla*I site of the pBR322 vector. After *Cla*I digestion, the *Cla*I site was made flush-ended with Klenow DNA polymerase I, and the flush-ended 1.5-kb *Eco*RI-*Hin*dIII fragment was inserted, placing the hsp70 promoter directly upstream from nt 40 (30) at the 5′ end of the P element coding sequences. This plasmid was digested with *Bal*I to produce an 8.0-kb fragment, and synthetic 8-bp *Bam*HI linkers (Collaborative Research) were added. After *Bam*HI cleavage, a 5.1-kb *Bam*HI fragment containing the hsp-P fusion gene but lacking functional P element 31-bp inverted repeats was purified and inserted into the pcopneo vector yielding pNHP.

Cell culture and DNA transformation. *D. melanogaster* Schneider line 2 cells were obtained from I. B. David and cultured as described previously (12). These cells were grown at 25°C in 25-cm^2 T-flasks (Corning Glass Works) to 1 $\times 10^6$ to 5 $\times 10^6$ cells per ml. The cells were seeded at ca. 0.5 $\times 10^6$ to 1 $\times 10^6$ cells per ml into 5 ml of medium in a 60-mm petri dish (Falcon Plastics) 12 h before transfection. Calcium phosphate-DNA coprecipitates were formed as previously described (12, 16, 51), and coprecipitate containing 10 μg of plasmid DNA was applied per 60-mm dish of cells. The cells were incubated in the presence of the calcium phosphate-DNA coprecipitate for 18 to 24 h at 25°C. The medium and DNA were removed, the cells were washed gently with phosphate-buffered saline, and fresh medium was added. After 24 h at 25°C, the culture medium was replaced with medium containing 1 mg of G-418 (GIBCO Diagnostics) per ml. G-418-resistant cells appeared after 7 to 12 days and were grown as mixed cell cultures. Cells treated with G-418 without DNA transfection were more than 95% killed and gave rise to only a very few slow-growing cells that were not characterized further. The G-418-resistant cells attached poorly to the culture dish, and hence single colonies of transformed cells could not be isolated. Initial attempts to clone the cells by limiting dilution were unsuccessul, and therefore the G-418-resistant cells were grown in mixed culture.

DNA analysis. High-molecular-weight DNA was isolated from Schneider line 2 cells or G-418-resistant transformants as described previously (32) and quantitated by measuring absorbance at 260 nm. The DNA was cleaved with restriction enzymes (New England Biolabs) and subjected to agarose gel electrophoresis and blot hybridization (24) with a ^{32}P-labeled probe prepared by nick translation (24). The probe contained cloned P element sequences (nt 40 through 2883) (30) in the plasmid vector pUC18 (47). Plasmid DNA was prepared by standard techniques (24).

RNA analysis. Total cellular RNA was prepared from tissue culture cells or adult fruit flies as described previously (29). Polyadenylic acid-containing [poly(A)$^+$] RNA was purified on oligodeoxythymidylate-cellulose (3), and 2 μg was subjected to gel electrophoresis in the presence of formaldehyde (24), transferred to nitrocellulose, and hybridized to ^{32}P-labeled M13-P element probes (30). These probes were prepared as described previously (29) and contained P element nt 40 through 878 and nt 1136 through 1378 (30). S1 nuclease mapping was carried out as described previously (6, 49). A ^{32}P-labeled, single-stranded, 347-nt *Hin*dIII-*Xba*I probe was prepared by labeling the *Hin*dIII site at the junction between the hsp70 and P element sequences in pNHP with [γ-^{32}P]ATP (ICN) and polynucleotide kinase (New England Biolabs), digesting it was *Xba*I, and separating the strands as described previously (24, 25). After hybridization and S1 nuclease digestion, the products were electrophoresed on 8% polyacrylamide–urea gels.

RESULTS

Construction of the pcopneo vector and a derivative carrying the hsp70 promoter-P element fusion gene. The bacterial neomycin resistance gene (*neo*) from transposon Tn*5* can be used as a dominant selectable marker for gene transfer in mammalian cells because it confers resistance to G-418, a derivative of gentamicin (11, 40). We reasoned that G-418 resistance could allow selection for gene transfer into cultured *D. melanogaster* cells. To ensure that the *neo* gene would be efficiently expressed after introduction into *D. melanogaster* cells, we put *neo* under the transcriptional control of the promoter in the LTR of the copia transposable element. This promoter is known to be efficiently utilized in *D. melanogaster* tissue culture cells (13). A 1.6-kb *Apa*I-*Eco*RI fragment carrying the copia LTR from plasmid cDm5002 (13) was ligated to a 2.5-kb *Hin*dIII-*Bam*HI fragment carrying the *neo* gene, as well as a simian virus 40 intron and polyadenylation signal from plasmid pSV2-neo (40), and inserted into the pAT153 plasmid vector (46) (see above). The resulting plasmid, called pcopneo, carried the copia LTR 5′ to the *neo* protein-coding region (Fig. 1). The vector contained unique *Eco*RI, *Bam*HI, and *Sal*I sites for the insertion of foreign DNA fragments.

To test the suitability of this system for the introduction and expression of nonselected DNA sequences in cultured *D. melanogaster* cells, we placed the coding sequences from the P transposable element (30) under the control of the 70-kilodalton heat shock protein (hsp70) promoter and inserted this fusion gene into the *Bam*HI site of pcopneo (Fig. 1) (see above). In this construct, called pNHP, the P element sequences lacked their normal promoter and should be under the transcriptional control of the hsp70 promoter, which is transcribed at high levels in *D. melanogaster* when the temperature is shifted from 25 to 37°C (2).

The vector plasmid pcopneo and its derivative pNHP were precipitated in the presence of calcium phosphate (12, 16, 51) and applied separately to cultures of *D. melanogaster* Schneider line 2 cells. After 24 h at 25°C, the cultures were transferred into selective medium containing 1 mg of G-418 per ml. G-148-resistant cells grew in 7 to 12 days from cultures that were treated with pcopneo or pNHP DNA. No G-418-resistant cells grew from mock-transfected cultures. Schneider line 2 cells attach poorly to culture flasks and do not form colonies. Thus, it was not possible to accurately determine the transformation frequency. Nevertheless, from the cell density at the time of transfection and the number of G-418-resistant cells obtained, we estimated a transformation frequency of ca. 10^{-4} to 10^{-5}, which is typical for experiments of this type carried out with mammalian cells. The lack of cell attachment to the substrate also precluded the cloning of individual cell colonies; thus, the G-418-resistant cells were grown en masse as mixed cultures. Our initial attempts to clone the cells by limiting dilution were unsuccessful.

Transformed DNA in G-418-resistant cells is present in tandem arrays. The presence and state of the introduced DNA in the G-418-resistant cell cultures was assayed by standard DNA blot hybridization analysis (39). High-molecular-weight DNA was isolated (32) from untransformed Schneider line 2 cells or from the G-418-resistant pcopneo- or pNHP-transformed cell lines. The DNA was

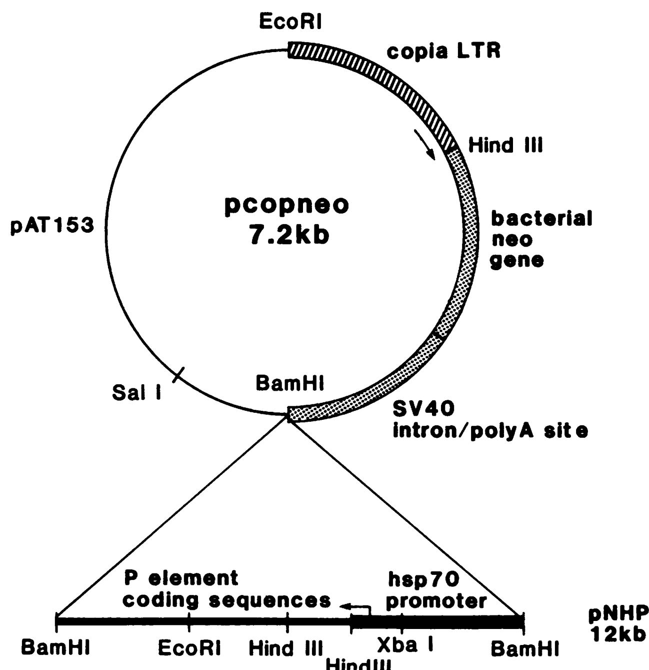

FIG. 1. Structure of the pcopneo vector and its derivative pNHP. The pcopneo vector was constructed by trimolecular ligation of *Eco*RI-*Bam*HI-cleaved pAT153 vector DNA, a 1.6-kb *Hin*dIII (*Apa*I)-*Eco*RI copia LTR-containing DNA fragment (13), and a 2.5-kb *Hin*dIII-*Bam*HI fragment carrying the bacterial *neo* gene. The pNHP derivative was made by inserting a 5.1-kb *Bam*HI fragment carrying the hsp-P fusion gene into the *Bam*HI site of pcopneo. The details of each construction are given in the text.

cleaved with endonuclease *Bam*HI, which cleaves once in the pcopneo vector and twice in the pNHP derivative (Fig. 1). After gel electrophoresis and blot hybridization with a ^{32}P-labeled DNA probe homologous to both pAT153 and the P transposable element (see above), autoradiography revealed a single 7.2-kb band of hybridization to DNA from the pcopneo transformants (Fig. 2A, lane 2). No hybridization was observed with DNA isolated from untransformed Schneider line 2 cells (lane 1). This result indicates that Schneider line 2 cells do not carry endogenous P element sequences. The single 7.2-kb band of hybridization seen with DNA from pcopneo transformants comigrated with pcopneo plasmid DNA linearized with *Bam*HI (lane 3). Similarly, the two bands of 7.2 and 5.1 kb from pNHP-transformed cell DNA (lane 4) comigrated with pNHP DNA cleaved with *Bam*HI (lane 5). These results indicate that the DNA within transformed G-418-resistant *D. melanogaster* cells is present in tandem head-to-tail arrays. The background hybridization observed in the transformed cell DNA samples presumably arose from junction fragments between vector and host DNA sequences which were heterogeneous in size, since these were not cloned cell lines. Furthermore, by knowing the amount of DNA present in each sample and by comparison with plasmid DNA standards, we calculated that there were an average of 10 to 20 copies of the vector DNA integrated in each transformed cell. Additional evidence that the DNA was integrated in tandem arrays was obtained by cleaving DNA from the transformed cells with a restriction enzyme that did not cleave the input vector DNA. Such digests should give rise to a unique band of hybridization corresponding to each site of insertion. All hybridization was confined to very-high-molecular-weight DNA (>50 kb) (Fig. 2B) (see below), consistent with integration of all 10 to 20 copies of the introduced DNA in a single tandem array in both pcopneo- and pNHP-transformed cells.

DNA introduced into G-418-resistant cells is stable in the absence of selection. The exogenous DNA found in the G-418-resistant, transformed *D. melanogaster* cell lines should, if integrated into chromosomal DNA, be stably maintained in the absence of G-418 selection. The vector DNA would then be replicated along with the host chromosomes irrespective of the presence or absence of selective drug (27, 40, 50–52). However, if the transformed DNA were episomal and not integrated into chromosomal DNA, then the foreign DNA sequences might be lost after the cell lines were grown without G-418 (45).

To ascertain whether the transfected vector DNA is stable without selection, high-molecular-weight cellular DNA was prepared from cultures of the pcopneo- and pNHP-transformed cell lines grown either with or without G-418 for 3 weeks at 25°C (ca. 20 to 25 cell generations). These DNA samples were cleaved with endonuclease *Apa*I and then analyzed by agarose gel electrophoresis and blot hybridization with a probe homologous to both the pcopneo vector and its derivative pNHP (see above). *Apa*I does not cleave either the pcopneo vector or pNHP, and therefore any band(s) arising from *Apa*I cleavage corresponds to an independent insertion site. *Apa*I digestion was confirmed by comparing the migration of genomic DNA samples in agarose gels before and after *Apa*I treatment. With DNA isolated from both the pcopneo- and pNHP-transformed cell lines (Fig. 2B, lanes 1 and 3), all hybridization was confined to very-high-molecular-weight (>50 kb) DNA fragments. Furthermore, no quantitative differences in hybridization were observed with DNA isolated from cells grown without (lanes 2 and 4) and with (lanes 1 and 3) G-418, implying that the exogenous DNA is stably integrated into and replicated along with the chromosomal DNA. These results suggest that the transformed vector DNA is integrated at a single or at most a few genomic locations and is stable for many generations in the absence of selection.

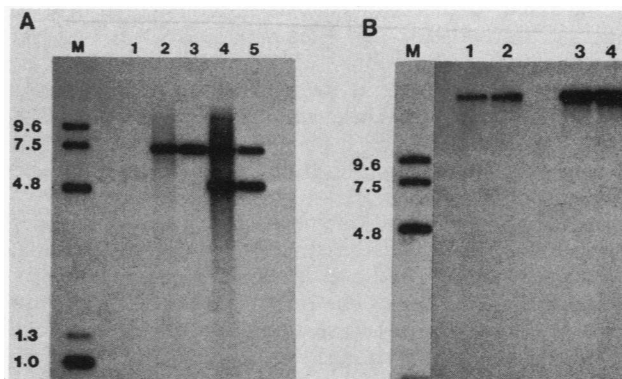

FIG. 2. Analysis of DNA from G-418-resistant cell lines. High-molecular-weight DNA was isolated and subjected to restriction enzyme cleavage and blot hybridization. (A) *Bam*HI cleavage. Lanes: 1, untransformed (control) Schneider line 2 cell DNA; 2, pcopneo-transformed cell DNA; 3, pcopneo DNA marker; 4, pNHP-transformed cell DNA; 5, pNHP DNA marker. (B) *Apa*I cleavage. Cells transformed with pcopneo (lanes 1 and 2) or pNHP (lanes 3 and 4) were grown with (lanes 1 and 3) or without (lanes 2 and 4) G-418 for 21 days (ca. 25 generations). The probe was pUC18, carrying cloned P element sequences (nt 40 through 2878 [30]). Fragment sizes (in kilobases) are indicated.

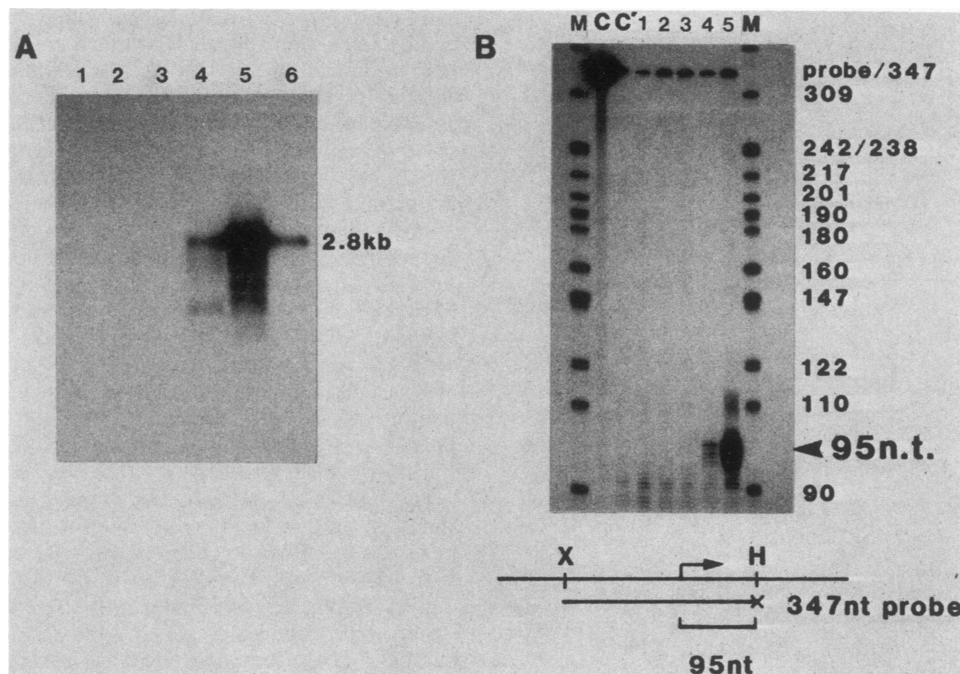

FIG. 3. Analysis of RNA from transformed *D. melanogaster* cell lines carrying the hsp-P fusion gene. RNA was isolated and subjected to RNA blot hybridization (A) or S1 nuclease analysis (B). Lanes 1, Untransformed Schneider line 2 cells; pcopneo-transformed cells grown at 25°C (lanes 2) or given a 37°C heat shock (lanes 3); and pNHP-transformed cells grown at 25°C (lanes 4) or given a 37°C heat shock (lanes 5). (A) Lane 6, RNA from heat-shocked *D. melanogaster* adults carrying the hsp-P fusion gene. The probe was a mixture of single-stranded M13 DNA homologous to P element nt 40 through 878 and nt 1136 through 1378 (30). (B) Controls were intact probe in the absence of RNA or S1 nuclease (lane C) or probe without RNA but treated with S1 nuclease (lane C'). Lanes M, *Msp*I fragments of ^{32}P-end-labeled pBR322 DNA (24) as markers. Fragment sizes (in nucleotides) are indicated. The diagram shows the 347-nt *Hin*dIII (H)-*Xba*I (X) probe and the 95-nt S1 nuclease-protected fragment corresponding to the 5′ portion of the RNA transcript.

Expression of the exogenous hsp70-P element fusion gene is induced by heat shock of pNHP-transformed cells. One useful application of this host-vector system is the overexpression of *D. melanogaster* gene products. To test this possibility, we put coding sequences from the P transposable element (30) under the control of the strong heat-inducible promoter for the major 70-kilodalton heat shock protein (hsp70) (2, 31) (Fig. 1). Since the normal P element mRNA is very rare [about 0.001% of the poly(A)$^+$ RNA] (18), the analysis of P element mRNA would be greatly facilitated by its overproduction. This hsp70-P element fusion gene is biologically active, since it provides P element transposase functions after *Drosophila* germline transformation (unpublished data).

Expression of the hsp-P element fusion gene in the pNHP-transformed cells was first assessed by RNA blot hybridization analysis (24). Total cellular RNA was prepared from untransformed Schneider line 2 cells grown at 25°C and from pcopneo- and pNHP-transformed G-418-resistant cells grown at 25°C or heat shocked for 1 h at 37°C before RNA isolation. Poly(A)$^+$ RNA was purified by oligodeoxythymidylate-cellulose chromatography and subjected to agarose gel electrophoresis under denaturing conditions, followed by blot hybridization with single-stranded ^{32}P-labeled P element DNA probes (see above). Autoradiography revealed a prominent 2.8-kb RNA species present in RNA samples from the pNHP-transformed cells (Fig. 3A, lanes 4 and 5). This RNA was induced at least 20- to 30-fold after a 1-h 37°C heat shock (lane 5), demonstrating transcriptional control characteristic of the heat shock promoter. After heat induction, the 2.8-kb RNA constituted ca. 0.5 to 1% of the poly(A)$^+$ RNA, as judged by comparison with actin mRNA levels (29) (data not shown). Furthermore, this 2.8-kb RNA was the size predicted from the vector construction and was the same size as that found in adult fruit flys transformed by using P element vectors with the hsp70-P element fusion gene (Fig. 3A, lane 6). In addition, RNA mapping experiments with S1 nuclease or RNase protection and the sequences of cloned cDNAs indicated that this 2.8-kb RNA was spliced in the same way as the P element mRNA isolated from embryos of a *D. melanogaster* strain containing a single full-length P element (F. Laski and D. Rio, unpublished data). No RNA homologous to the P element probe was found in untransformed Schneider line 2 cells (Fig. 3A, lane 1) or in cells transformed with the pcopneo vector grown at 25°C (lane 2) or after a 1-h 37°C heat shock (lane 3). Moreover, when a parallel RNA blot was probed with a *neo* gene probe, a 2.4-kb RNA corresponding to the copia-*neo* gene transcript was found in pcopneo- and pNHP-transformed cells but not in untransformed control cells (data not shown).

The 5′ terminus of the 2.8-kb hsp-P transcript was mapped by S1 nuclease protection analysis (6, 49). A 347-nt *Hin*dIII-*Xba*I single-stranded DNA probe was prepared by labeling the 5′ end of a *Hin*dIII site, located in the P element sequences 95 bp from the expected 5′ terminus of the hsp-P element transcript (31), with ^{32}P. This probe was specific for the fusion gene. Although the probe will hybridize both to the hsp-P element mRNA and to endogenous hsp70 transcripts, the portion of the probe containing the 5′ ^{32}P label was derived from P element sequences and thus would only be protected from S1 nuclease digestion by hybridization to

the hsp-P element fusion gene transcript. A protected DNA fragment of 95 nt was observed only when the probe was annealed to RNA isolated from pNHP-transformed cells (Fig. 3B, lanes 4 and 5). Furthermore, the amount of this S1-resistant product increased dramatically (ca. 20- to 30-fold) when the cells were heat-shocked for 1 h at 37°C before RNA isolation (lane 5) relative to the amount found with RNA from cells grown at 25°C (lane 4). Moreover, no 95-nt S1-resistant product was found in RNA from pcopneo-transformed cells grown at 25°C (lane 2) or heat-shocked for 1 h at 37°C (lane 3), from untransformed Schneider line 2 cells grown at 25°C (lane 1), or in the absence of RNA (lane C'). These results indicate that the 2.8-kb hsp-P element fusion gene transcript initiates, as expected, from the hsp70 promoter in the pNHP-transformed cells and that this transcript is induced to high levels after heat shock.

DISCUSSION

We describe the construction and functional analysis of a vector that allows the stable transformation of *D. melanogaster* Schneider line 2 tissue culture cells. This vector carries the bacterial neomycin resistance gene under the transcriptional control of the copia transposable element promoter and confers resistance to G-418, thus serving as a dominant selectable marker for the introduction of foreign DNA sequences into the genomes of cultured *D. melanogaster* cells. The introduced DNA is apparently integrated in tandem arrays of 10 to 20 copies per cell and is stable in the absence of selection. Interestingly, the exogenous DNA appears to be integrated at a single or at most a few locations and apparently lacks the gross deletions and rearrangements commonly found to occur with DNA transformed into mammalian cells (14, 33).

Several other vectors for *D. melanogaster* tissue culture transformation have been described (8, 38). The use of one such vector, which relies on methotrexate resistance for selection, results in transformed cells that carry more than 1,000 copies of the foreign DNA sequences (8; P. Cherbas, personal communication). It is unclear whether this large amount of DNA is integrated or maintained extrachromosomally. Another transformation vector uses the *Escherichia coli* guanine phosphoribosyltransferase (*gpt*) gene as a selectable marker (38). However, in this case the DNA is maintained extrachromosomally by an as yet undefined mechanism (38). Both of these systems require specially formulated selective media.

The transformation system we described here offers several advantages over those previously described. First, the transformed DNA is present in a relatively low copy number (10 to 20 copies per cell). Second, the DNA appears to be unrearranged and to be integrated into the chromosomal DNA, Third, no special medium is required for this selection; G-418 is simply added to the standard culture medium.

A variety of applications can be seen for this system. We have shown that the pcopneo vector can be used to introduce unselected DNA (the hsp-P element fusion gene) and that transcription of this DNA is induced by heat shock. The hsp70 promoter might be useful for the expression of protein products from heterologous genes, since the 90-nt heat shock leader RNA coded for in the hsp70 promoter DNA fragment is apparently able to confer heat shock translational control (7, 12). Furthermore, we have shown that after heat shock, the RNA from the hsp-P fusion gene in transformed tissue culture cells is spliced in the same way as that found in a fly strain carrying a single full-length P element (F. Laski and D. Rio, unpublished data). We infer that the 2.4-kb poly(A)$^+$ RNA complementary to the *neo* gene probe seen on RNA blots from our G-418-resistant transformed cells encodes the aminoglycoside phosphotransferase APH(3')II (40). This implies that the polypeptide possessing the phosphotransferase enzymatic activity is being expressed, since G-418 resistance involves aminoglycoside phosphorylation by this enzyme. We are currently investigating the nature of the P element polypeptides encoded by the hsp-P element fusion gene, as well as other P element constructs. Thus, this system could be used to produce a number of important regulatory gene products from *D. melanogaster* for isolation and biochemical characterization. Furthermore, it should serve to complement transient expression assays carried out in cultured *D. melanogaster* cells (5, 9, 12) as well as P element-mediated germline transformation (34, 42) in the study of *D. melanogaster* gene regulation.

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