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The white gene as a marker in a new P-element vector for gene transfer in *Drosophila*

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ABSTRACT

We describe new vectors suitable for P-element mediated germ line transformation of Drosophila melanogaster using passenger genes whose expression does not result in a readily detectable phenotypic change of the transformed flies. The P-element vectors contain the white gene fused to the heat shock protein 70 (hsp70) gene promoter. Expression of the white gene rescues the white$^-$ phenotype of recipient flies partly or completely even without heat treatment. Transformed descendents of most founder animals (G0) fall into two classes which are distinguishable by their orange and red eye colours. The different levels of white expression are presumably due to position effects associated with different chromosomal sites of insertion. Doubling of the gene dose in orange eyed fly stocks results in an easily visible darkening of the eye colour. Consequently, the generation of homozygous transformants is easily possible by simple inbreeding due to the phenotypic distinction of homo- and heterozygous transformants. Cloning into these P-element vectors is facilitated by the presence of polylinkers with 8 and 12 unique restriction sites.

INTRODUCTION

The introduction of genes into the germ line of Drosophila melanogaster by means of P-factor mediated transformation usually occurs with high frequency, i.e. a high proportion of flies arising from injected embryos (G0) give rise to some transformed offspring (G1). The number of transformants among the offspring of different G0 flies varies considerably from about 40% to values as low as 0.5% (1). Only a few genes convey in an easily recognizable phenotype to the recipient fly stock. For those which do not one can avoid laborious screening by linking the gene of interest to a marker gene which manifests itself distinctly in transformants. Marker genes which have been used for that purpose confer either a selectable or a visible phenotype to transformants. The use of the Drosophila alcohol dehydrogenase gene (2) and the bacterial neomycin resistence gene (3) allow selection of the transformants on ethanol and the antibiotic G418 respectively. Marker genes which confer a visible phenotype to transformants have the advantage of being easily followed in subsequent generations. The most frequently used marker of this kind is the rosy gene (ry) which encodes xanthine dehydrogenase (1,4). The eye color of homozygous ry $^-$ flies is dark crimson and it is reverted to the brick-red wild type colour in transformants. The major drawbacks of the rosy gene as a

marker are the availability of only three restriction sites (SalI, XbaI, HpaI) for cloning purposes (4,5) and the subtle phenotype of the mutation. The white mutation on the other hand has a very strong phenotype. Homozygous null mutants have bleach white eyes and the wild type colour can be rescued by transformation with the white gene (6-10).

We have constructed P-element vectors in which a truncated white gene is fused to the hsp70 gene promoter. The construct was designed such that many hexameric restriction enzyme recognition sites were eliminated. Polylinkers with 12 and 8 unique restriction sites were added up- and downstream of the hsp70 gene. The transformation frequency with these constructs is very high. Many of the transformed flies are orange rather than red eyed in the absence of a thermal shock. Doubling of the gene dose is manifested by an easily visible darkening of the eye colour. This phenotypic distinction between hemi- and homozygous transformants allows one to generate homozygous transformed lines by simple inbreeding.

MATERIALS AND METHODS

Plasmid Construction. The source of the white gene was plasmid pWP-2 (7) which contains a 12 kb EcoRI fragment from phage M558 (11) inserted between the ends of a P-element. The sequence of 14 kb from the white locus has been determined by O'Hare et al. (12) and position numbers used throughout this report refer to their publication.

The 535 bp MaeI, HindIII fragment located between positions +3707 and +3172 which contains 268 pb of the 5' terminal part of the first intron and, according to Pirrotta and Brockl (13) most and according to Steller and Pirrotta (14) all of the non-translated leader region was isolated and the ends were made blunt ended by treatment with the Klenow fragment of E. coli DNA polymerase I. This restriction fragment was cloned into the SalI site of pUC18 whose sticky ends have been filled in with the Klenow enzyme to give rise to plasmid pW1 (Fig. 1B).

In order to shorten the 3' non transcribed flanking region of the white gene 2.2 kb around the KpnI site at the approximate position -4400 were removed by treatment with Bal31 exonuclease (Fig. 1). The XbaI, EcoRI fragment of the resulting plasmid was inserted between the XbaI and EcoRI sites of pW1 (Fig. 2C). The plasmid pW2 was converted to pW3 by linearizing it with XbaI, filling in the sticky ends with the Klenow enzyme and religating the plasmid, thereby destroying the XbaI site (Fig. 2D).

A 388 bp HaeIII-XmnI fragment that contains 177 bp of promoter and 216 bp of leader sequence of the hsp70 gene was isolated from pHSL (8), a derivative of plasmid 56H8 (15) which contains a hsp70 gene from the chromosomal region 87C. This fragment was cloned into the PstI site of pW3 whose 3' overhangs have been removed with T4 DNA polymerase (Fig. 2E). The HindIII, EcoRI fragment of the resulting plasmid pW4 was isolated, the sticky ends made

blunt ended by treatment with the Klenow fragment of E.coli DNA polymerase and ligated into the filled in HindIII site in the polylinker of the plasmid car4.3 (Fig. 2F) which was also made blunt ended with the Klenow enzyme. This latter plasmid was derived from the P-element containing construct car4 (4) by inserting XhoI linkers (CCTCGAGG) into the filled in SalI site, a KpnI linker (GGGTACCC) into the T4 DNA polymerase treated SacI site and a BglII linker (CAGATCTG) into the filled in BamHI site. In the resulting plasmids pW5 (Fig. 2G) and pW6 (Fig. 2H) the hsp70-white fragment was inserted such that the polylinker is upstream and downstream of the hsp70 promoter respectively. pW5 was linearized with XhoI, and the synthetic HpaI linker (GTTAAC) (Pharmacia) was ligated to the filled in XhoI ends (Fig. 2I). A synthetic polylinker containing the recognition sites for the enzymes NotI, SacII, SfiI and SpeI (Boehringer Mannheim, Biochemica) was inserted into the BglII site of pW7 (Fig. 2K). A 388 bp EcoRI, PvuII fragment spanning the polylinker and the hsp70 promoter region was isolated from this final construct pW8 and was inserted into the EcoRI and SmaI sites of M13 mp18 and M13 mp19. The hsp70 sequences of the EcoRI, PvuII fragment in M13 mp18 were eliminated because the pW8 polylinker sequence was too far away from the sequencing primer for accurate sequence determination. This could easily be performed by cleaving at the two SphI sites in the M13 mp18 and the pW8 polylinker followed by religation. Thereby a M13 vector was created with the additional unqiue recognition sites for the enzymes SacII, NotI, SfiI, SpeI, XhoI, StuI and HpaI but lacking the SacI site. Sequencing of both strands of the pW8 polylinker was done according to Sanger et al. (16). The sequence reveals the insertion of 2 XhoI and 4 HpaI linkers. One of the central HpaI linkers, however, lacks a T (Fig. 3, position 95). Insertions of the BglII and the HpaI linkers into the BamHI and XhoI sites respectively should have duplicated the BamHI and the XhoI sites. One of each of these sites got lost due to a deletion of the terminal nucleotide of the left hand BamHI site (Fig. 3, position 24) and an insertion of the AC dinucleotide of the left hand XhoI site (Fig. 3, position 80 and 81).

The 5.5 kb XbaI-EcoRI fragment from p75RH (17) containing the indirect flight muscle specific actin gene with a nonsense mutation at position Trp356 (18,19) was inserted between the EcoRI and the XbaI sites of pW5 to give rise to pWAc. The inserted fragment contains 1.4kb and 1.7 kb of 5′ and 3′ flanking sequence respectively. The white and the actin genes in pWAc are transcribed in opposite orientations.

Injection of Embryos. The fly strain w^1, sn^w/w^1, sn^w; st/+ served as the recipient in the transformation experiments. Embryos were mechanically dechorionated and injected according to Spradling and Rubin (20,1) with 300 µg/ml of pW5 or pW6 and 100 µg/ml pπ 25.7 wc (21). Plasmid pW5 and pW6 DNA was purified by preparing a cleared supernatant, as described by Clewell and Helinski (22) followed by banding in CsCl-ethidium bromide gradients. Plasmid pWAc DNA was prepared according to the rapid small scale isolation

procedure of Holmes and Quigley (23). The DNA was bound to an elutip column (Schleicher and Schüll) following RNaseA treatment and phenol extraction, eluted with 1 M NaCl, 20 mM Tris HCl, pH 7.5, 1 mM EDTA and concentrated by ethanol precipitation. Serial dilutions of the purified plasmid DNA were run on a 1% agarose gel in parallel with a serial dilution of a plasmid preparation of known concentration in order to estimate the pWAc DNA concentration.

In situ hybridization to polytene chromosomes. In situ hybridization to polytene chromosomes was carried out with biotinylated probes according to the method of Langer-Safer et al. (24). Materials used for this procedure were bio-UTP with an 11-carbon linker arm, synthesized by M. Mlodzik as described by Langer et al. (25) with minor modifications, goat anti biotin IgG (Enzo-Biochemicals), rabbit anti-goat antibody conjugated to horseradish peroxidase (Dakopads), and the DNA probe described by Gehring et al. (7).

Analysis of IFM actin. Adult flies were injected with ^{35}S-methionine (30 mCi/ml) within 1 hour after eclosion, aged for two hours at room temperature and then freeze-dried in an acetone/dry ice mixture (26). IFM were dissected from dehydrated flies, dissolved in SDS sample buffer and the proteins separated on a 12% SDS polyacrylamide gel (27).

RESULTS AND DISCUSSION

Construction of the hsp70-white P-element vectors

In previous transformation experiments we and others were able to rescue white$^-$ mutations with a 12 kb fragment of genomic DNA (6,7). Later we used the white gene as a marker to follow the integration of a heat shock gene into the Drosophila genome (28). In all these experiments transformed flies were obtained with only very low frequencies. Since the transformation frequency seems to depend upon the size of the vector (4) we decided to reduce the size of the white gene to obtain a more suitable transformation vector. Secondly, in order to obtain a vector which is suitable for the easy insertion of DNA fragments a number of hexameric restriction enzyme recognition sites within the white gene were eliminated and added back to the construct in a polylinker up- or downstream of the white gene.

2.2 kb of the 3′ flanking sequence around the KpnI site at the approximate position -4400 (Fig. 1) were first removed by treatment with the exonuclease Bal 31. The deletion endpoints were not precisely determined but must be very close to the SalI (-3050) and EcoRI (approximately - 5300) sites as those sites are left intact and are now only 210 bp apart. Subsequently, the size of the largest intron was reduced to 356 bp by deleting the 2.7 kb XbaI/HindIII fragment. Finally a 388 bp fragment from the hsp70 heat shock gene, containing all the necessary sequences for efficient transcription in flies (29,30) and most of the leader sequence was fused to the MaeI site at position +3708. If the original mapping of the first exon of the white gene were correct (13) this MaeI site would be located within the non translated

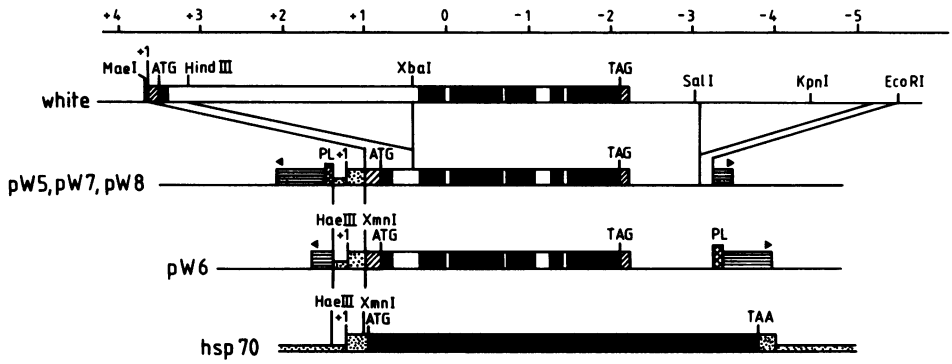

Figure 1

The hsp70-white P-elements. The hsp70-white P-elements, arbitrarily cut at the PvuI site in the car4 sequence, are shown in the middle, the white gene above and the hsp70 gene below them. Coding sequences and introns are indicated with filled and empty boxes respectively, non translated leader and trailer sequences of the white gene with hatched and of the hsp70 gene with dotted boxes. The two halves of the P-element are shown as horizontally striped boxes and the 31 bp inverted terminal repeats as arrowheads. The polylinkers (PL) are represented by the cross hatched boxes. Only restriction sites relevant for the construction are indicated. The transcription initiation site (+1) of the white gene is taken from Steller and Pirrotta (14). The zero point in the coordinate scale above the maps indicates the site of the copia element insertion in the w^a mutant. The size units are given in 1 kb.

A)	R, Sa, K, Sm, B, X, S, P, Sp, H	pUC18
B)	R, Sa, K, Sm, B, X [II] P, Sp, H	pW1
C)	R [III] X [II] P, Sp, H	pW2
D)	R [III] [II] P, Sp, H	pW3
E)	R [III] [II] [I] Sp, H	pW4
F)	R, K, Sm, Bg, B, X, S, Xh, St, Xh, S, P, H	car4.3
G)	R, K, Sm, Bg, B, X, S, Xh, St, Xh, S, P, Sp [I] [II] [III]	pW5
H)	R, K, Sm, Bg, B, X, S, Xh, St, Xh, S, P [III] [II] [I] Sp	pW6
I)	R, K, Sm, Bg, B, X, S, Hp, Hp, Hp, Xh, St, Xh, S, P, Sp [I] [II] [III]	pW7
K)	R, K, Sm, Sc, N, Sf, Spe, B, X, S, Hp, Hp, Hp, Xh, St, Xh, S, P, Sp [I] [II] [III]	pW8

Figure 2

Construction of hsp70-white P-elements. The restriction sites in the polylinkers of pUC18 (A) and car4.3 (F) as well as in their derivatives are indicated. Restriction sites in the polylinkers of pW6 and pW8 which do not appear at other locations within these plasmids are underlined. The boxes represent: the HaeIII, PvuII fragment from the hsp70 gene (I); the MaeI, HindIII fragment from the leader and first intron region of the white gene (II), and the XbaI, EcoRI fragment containing the bulk of the white gene (III). The boxes are not drawn to scale.

B : BamHI, Bg : BglII, H : HindIII, Hp: HpaI, K : KpnI, N: NotI, P : PstI, R : EcoRI, S : SalI, Sa : SacI, Sc: SacII, Sf: SfiI, Sm : SmaI, St: StuI, Sp : SphI, Spe: SpeI, X : XbaI, Xh : XhoI.

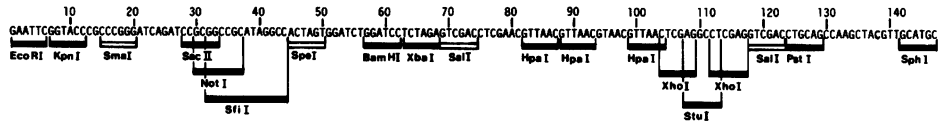

Figure 3
Sequence of the polylinker in pW8. Unique and non unique restriction sites are indicated with filled and empty boxes respectively.

leader sequence very near the transcription start site. However, Steller and Pirrotta recently mapped the 5′ end of the mRNA further downstream to position 3689 $^{+}$ 2 (14). Indeed, the sequence TTCAGTT between the positions +3684 and +3690 fits the consensus heptanucleotide sequence ATCA^{G}/T^{C}/T found at the 5′ ends of most insect mRNAs in 6 out of 7 positions (31). Accordingly, the Mael site would be located 18 bp upstream of the transcription initiation site. By replacing the white promoter with the short hsp70 promoter we could further decrease the size of the vector and eliminate more restriction sites.

The two plasmids pW5 and pW6 (Fig. 1, Fig. 2G,H) were obtained by inserting this fusion construct into the P-element containing plasmid car4.3 which is a derivative of car4 (4) with an extended polylinker (Fig. 2F). pW8 was derived from pW5 by the addition of the recognition sites for the restriction enzymes HpaI, NotI, SfiI, SacII and XhoI to the polylinker (Fig. 2 I,K). The white vectors are 8.3 kb long, 4.6 kb of which are between the ends of the P-element and can be integrated into the Drosophila genome. The sequence of the entire plasmid is known (1,12,32,33) except for 210 bp between the SalI site at -3050 and the P-element. The polylinker of pW8 consists of twelve unique restriction sites (Fig. 3, Table I) and the one of pW6 has eight unique sites.

Transformation

The plasmid pW5 was coinjected with the P-factor helper plasmid pπ 25.7 wc (21) into embryos of a white eyed fly strain with the mutations w^{1} and sn^{w}. 29 out of 68 fertile GO flies, i.e. flies which arose from injected embryos, gave rise to non-white-eyed offspring. This transformation frequency is much higher than the one observed with the complete white gene. The eye pigmentation of the transformed flies was variable, ranging from very light orange to wild type brick red. Non homogeneous pigment deposition (variegation) was observed in two cases. In general males had more strongly pigmented eyes than females which might be due to dosage compensation, a term used to indicate that males overexpress X chromosomally located genes (like the white gene) with respect to females to compensate for the lower gene dose. This would mean that sequences required for dosage compensation are located within or downstream of the white gene which contrasts the finding of Pirrotta et al. (10) that sequences between -216 and the transcription start site are instrumental for this effect. The eye pigmentation

Table I Restriction Sites in pW8 Polylinker

Restriction Sites	Acceptor Sites	Recognition Sequences
Bam HI	BamHI	G|G A T C C
	BglII	A|G A T C T
	BclI	T|G A T C A
	XhoII	Pu|G A T C Py
	MboI	|G A T C
EcoRI	EcoRI	G|A A T T C
HpaI	blunt ends	
KpnI	KpnI	G G T A C|C
NotI	NotI	G C|G G C C G C
	XmaIII	C|G G C C G
	CfrI	C|G G C C G
		C|G G C C A
		T|G G C C G
		T|G G C C A
PstI	PstI	C T G C A|G
	NsiI	A T G C A|T
Sac II	SacII	C C G C|G G
SfiI	SfiI	G G C C N N N N|N G G C C
StuI	blunt	
SphI	SphI	G C A T G|C
	NlaIII	C A T G|
XbaI	XbaI	T|C T A G A
	AvrII	C|C T A G G
	NheI	G|C T A G C
	SpeI	A|C T A G T
XhoI	XhoI	C|T C G A G
	AvaI	C|PyC G Pu G
	SalI	G|T C G A C

a) Only those sites are listed which are unique in the plasmid

developed without heat treatment of the pupae. Steller and Pirrotta have recently described a similar hsp70-white fusion gene whose expression was obvious at normal temperature as well (14). Heat treatment of pupae from most strains led to predominantly red eyed offspring. However, only dark orange but no red eyed flies were found among the offspring of very pale

Table II **Crosses of Offspring of Orange Eyed Transformants**

Strain[a]	crosses[b]											
	or x or			r x r			or x w			r x w		
	offspring			offspring			offspring			offspring		
	w	or	r	w	or	r	w	or	r	w	or	r
2	27.9[c]	52.8	19.3	0	0	100	47.4	51.5	1.1	0	99.5	0.5
15	13.6	72.7	13.7	0	0	100	52.5	47.5	0	0	100	0
18	25.9	54.2	19.9	0	0	100	64.4	35.6	0	0	100	0
26	17.8	56.7	25.5	0	0	100	51.6	48.4	0	0	100	0
32	20.2	59.3	20.5	0	0	100	46.7	53.3	0	0	99.6	0.4
36	13.3	86.1	0.6	0	0	0[f]	51.2	48.8	0	0	0	0[f]
59	15.1	55.6	29.3	0	0	100	51.4	48.6	0	0	100	0
60	33.3	51.5	15.2	0	0	0[f]	41.2	58.8	0	0	100	0
67	34.9	63.5	1.6[d]	0	0	0[f]	48.8	51.2	0	0	0	0[f]
74	28.3	57.5	14.2	0	0	100	55.7	44.3	0	0	100	0
76A[e]	22.0	53.7	24.3	0	0	100	56.7	43.3	0	0	100	0
76B[e]	29.9	57.7	12.4	0	0	100	43.6	55.5	0.9	ND		

a) All strains are derived from orange eyed G1 flies
b) Abbreviations: w = white, or = orange, r = red
c) Values are expressed as percentage of the hatched flies
d) The majority of red eyed offsprings were incapable of emerging from the pupal case.
e) Strains 76A and 76B are from two phenotypically distinct offsprings of the same G0 fly. There is a gradient of eye pigmentation in strain 76A with the anterior part of the eye being orange and the posterior part white. The eyes of strain 76B are homogenously orange.
f) Homozygous semilethal and/or sterile.
ND: not done

orange parents even after extensive heat treatment of the pupae. This is a surprising finding in view of the fact that a duplication of the gene dose is sufficient to convert most orange eyed to red eyed strains (see below; Table II). The substantial increase in the hsp70 promoter strength after heat induction should be sufficient to render orange eyed strains red eyed. We therefore assume that there is post transcriptional down regulation.

The white$^+$ phenotype was not detected in G0 flies. The same has previously been observed in experiments where the white gene with its own promoter was used for transformation (7).

Table III **Chromosomal Location of the hsp70 white P-element**

strain	Eye colour of the G1 fly [a]	Chromosomal location
2	or	3L, 66A
	r	3L, 68B; 3R, 85C
15	or	2R, 57A
	r	2R, 50E/F
	r[b]	X, 7E/F
32	or	3R, 84/85
	r	3R, 86D/E
60	or	3L, 73B
	r	3L, 79E/F
63	or	3L, 73B
	r	3L, 75B
74	or	3R, 87 C/D
	r	3R, 90A

a) The orange and red colours are designated or and r respectively
b) The bristles are sn^e

Red- and orange eyed offspring of the same GO fly have P-element insertions at different locations

Red and orange eyed G1 flies were first backcrossed to the recipient strain. The eye colour of most of the transformed flies arising from these crosses corresponded to the one of the transformed parent. The red and orange eye colour phenotype thus represents a true genetic trait. However, we found a few wild type eye coloured flies in some crosses between orange and white eyed flies (Table II). These exceptional flies might have been exposed at the pupal stage to stress conditions during the critical period of the white gene expression.

If position effects are the cause for the different degree of eye pigmentation leading to red and orange eyed flies, the orange and red eyed progeny of one GO fly should contain P-element insertions at different locations. To test this hypothesis we determined the site of the P-element insertion in pairs of strains, one red and one orange eyed, arising from the same GO founder animal, by in situ hybridization to polytene chromosomes. Indeed, in all six pairs tested the P-element was found at different chromosomal locations in the orange and red eyed strains (Table III).

The red eyed animals of one of the strains (strain 15) fall into two classes, those with the same singed weak (sn^w) bristle phenotype as the recipient strain and others with singed

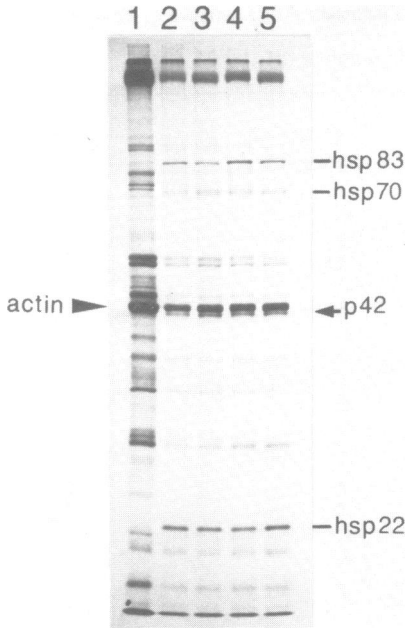

Figure 4
Proteins synthesized in the indirect flight muscles (IFM) of pWAc transformants and the recipient fly strain. Hemizygous females were injected with ^{35}S-methionine and aged for 2 hours. IFM was dissected from freeze-dried flies and electrophoresed on a 12% SDS PAGE. Lane 1 of the autoradiograph: the recipient fly strain. Lane 2: transformant EE123 (red eyed). Lane 3: EE117 (orange eyed), Lane 4: EE115 (orange eyed), Lane 5: EE96 (red eyed).

Actin and the mutant actin p42 are indicated by the arrowhead and the arrow respectively. Further indicated are the heat shock proteins (hsp) whose expression is turned on as a consequence of p42 synthesis (35).

extreme (sn^e) bristles. The hsp70-white P-element in the latter strain was found on the X chromosome at 7E/F, the site of the sn locus. It is therefore likely that the hsp70-white P-element insertion is the cause for the sn^e phenotype. The sn locus has previously been shown to be a hot spot for P-element transposition (20).

The hsp70-white P-element as a vehicle for gene transfer

To demonstrate the usefullness of the hsp70-white P-element as a transformation vehicle, the gene encoding an indirect flight muscle (IFM) specific actin was inserted between the XbaI and EcoRI sites in the polylinker of pW5. This actin gene has been isolated from a flightless mutant strain (34). A nonsense mutation in this gene leads to the accumulation of a truncated form of IFM specific actin and was shown to be the cause for the inability of these mutants to fly (17).

Four transformants, two orange and two red eyed, were tested for the synthesis of the truncated actin by metabolic labeling of proteins (Fig. 4). In all four transformants we could detect similar amounts of the mutant actin in the IFM. The constitutive synthesis of a subset of heat shock proteins in the IFM which results from the accumulation of truncated actin (35) is observed in all four transformants studied here.

Possible interference from the hsp70 enhancer might be reduced by cloning into plasmid pW6 which has also been successfully used to transform white eyed flies.

A simple strategy to generate homozygous transformants

The generation of flies homozygous for a transformed gene is usually a laborious task because hemi- and homozygous flies are phenotypically indistinguishable. In contrast, the hsp70-white hybrid gene is often expressed in hemizygotes at too low a level to allow for wild type eye pigmentation and the doubling of the gene dose is reflected in darker eye colours. Orange eyed individuals of twelve different strains were pair mated and some red or very dark orange offpring were obtained (Table II). Those were shown to be homozygous for the transformed gene: if inbred over many generations they only give rise to red eyed offspring and if crossed to the recipient fly strain all progeny are orange eyed. Orange eyed flies on the other hand are hemizygous for the transformed gene. Backcrossing to the recipient strain gives rise to 50% white and 50% orange eyed progeny (Table II).

The fact that the percentage of red eyed offspring in crosses between orange eyed flies most often is lower than the expected 25% must reflect reduced viability of homozygous transformants.

Darker eye colors in homozygous flies might be due to pairing of the hsp70-white gene (transvection) or it might be a simple dosage effect. We showed that the latter is the case by crossing two homozygous stocks with the hsp70-white gene on the second and the third chromosome. The offspring which still have two copies of the hsp70-white gene but unlike their parent on different chromosomes were still all red eyed.

The phenotypic distinction between homo- and hemizygous transformants allows one to generate homozygous transformants in only two generations and without the help of balancer chromosomes. Orange eyed G1 flies are first amplified by backcrossing them to the recipient fly stock and the transformed progeny are subsequently pair mated leading to some red eyed, homozygous transformants. This scheme can be of wide use as the fraction of orange eyed G1 flies is very high. We found orange eyed flies among the offspring of all but one GO flies that gave rise to transformed progeny (28/29). The generation of homozygous red eyed G1 flies has to be done according to the standard procedure involving appropriate balancer chromosomes (36).

Since it is easier to produce homozygous transformants from orange rather than red eyed lines, one is tempted to exclusively work with this category of flies thereby selecting for low expression of the white gene. This does not necessarily mean that one is selecting for strains with lower expression of the gene of interest as demonstrated in the previous section for the IFM actin gene.

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REFERENCES

1. Rubin, G.M. and Spradling, A.C. (1982) Science **218**, 348-353.
2. Goldberg, D.A., Posakony, J.W. and Maniatis, T. (1983) Cell **34**, 59-73.
3. Steller, H. and Pirrotta, V. (1985) The EMBO J. **4**, 167-171.
4. Rubin, G.M. and Spradling, A.C. (1983) Nucl. Acids Res. **11**, 6341-6351.
5. Simon, J.A., Sutton, C.A., Lobell, R.B., Glaser, R.L. and Lis, J.T. (1985) Cell **40**, 805-817.
6. Hazelrigg, T., Levis, R. and Rubin, G.M. (1984) Cell **36**, 469-481.
7. Gehring, W.J., Klemenz, R., Weber, U. and Kloter, U. (1984) The EMBO J. **3**, 2077-2085.
8. Klemenz, R., Hultmark, D. and Gehring, W.J. (1985) The EMBO J. **4**, 2053-2060.
9. Levis, R., Hazelrigg, T. and Rubin, G.M. (1985) The EMBO J. **4**, 3489-3499.
10. Pirrotta, V., Steller, H. and Bozzetti, M.P. (1985) The EMBO J. **4**, 350l-3508.
11. Goldberg, M.L., Paro, R. and Gehring, W.J. (1982) The EMBO J. **1**, 93-98.
12. O'Hare, K., Murphy, C., Levis, R. and Rubin, G.M. (1984) J. Mol. Biol. **180**, 437-455.
13. Pirrotta, V. and Brockl, Ch. (1984) The EMBO J. **3**, 569-568.
14. Steller, H. and Pirrotta, V. (1985) The EMBO J. **4**, 3765-3772.
15. Schedl, P., Artavanis-Tsakonas, S., Steward, R., Gehring, W., Mirault, M.-E., Goldschmidt-Clermont, M., Moran, L. and Tissières, A. (1978) Cell **14**, 921-929.
16. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA **74**, 5463-5467.
17. Hiromi, Y., Okamoto, H., Gehring, W.J., and Hotta, Y. (1986) Cell **44**, 293-301.
18. Karlik, C.C., Coutu, M.D. and Fyrberg, E.A. (1984) Cell **38**, 711-719.
19. Okamoto, H., Hiromi, Y., Ishikawa, E., Yamada, I., Isoda, K., Maekawa, H. and Hotta, Y. (1986) The EMBO J. **5**, 589-596.
20. Spradling, A.C. and Rubin, G.M. (1982) Science **218**, 341-347.
21. Karess, R.E. and Rubin, G.M. (1984) Cell **38**, 135-146.
22. Clewell, D.B. and Helinski, D. (1970) Biochemistry **9**, 4428-4440.
23. Holmes, D.S. and Quigley, M. (1981) Anal. Biochem. **114**, 193.
24. Langer-Safer, P.R., Levine, M. and Ward, D.C. (1982) Proc. Natl. Acad. Sci. USA **79**, 4381-4385.
25. Langer, P.R., Waldrop, A.A. and Ward, D.C. (1981) Proc.Natl. Acad. Sci. USA **78**, 6633-6637.
26. Mogami, K., Fujita, S.C. and Hotta, Y. (1982) J. Biochem. **91**, 643-650.
27. Lämmli, U.K. (1970). Nature **227**, 680-685.

28. Klemenz, R. and Gehring W. (1986) Mol. and Cell. Biol. **6**, 2011-2019.
29. Dudler, R. and Travers, A.A. (1984) Cell **38**, 391-398.
30. Amin, J., Mestril, R., Lawson, R., Klapper, H. and Voellmy, R. (1985) Mol. and Cell. Biol. **5**, 197-203.
31. Hultmark, D., Klemenz, R. and Gehring, W.J. (1986) Cell **44**, 429-438.
32. Ingolia, T.D., Craig, E.A. and McCarthy, J.M. (1980) Cell **21**, 669-679.
33. Karch, F., Török, I. and Tissières, A. (1981) J. Mol. Biol. **148**, 219-230.
34. Mogami, K. and Hotta, Y. (1981) Mol. Gen. Genet. **183**, 409-417.
35. Hiromi, Y. and Hotta, Y. (1985) The EMBO J. **4**, 1681-1687.
36. Karess, R. E. (1985). in DNA cloning, Vol. **II**, Glover D.M. ed. IRL Press, Oxford, pp.121-140.