Copy number control of a transposable element, the *I* factor, a LINE-like element in *Drosophila*

(transposition/regulation/cosuppression)

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ABSTRACT The *I* factor is a LINE-like transposable element in *Drosophila*. Most strains of *Drosophila melanogaster*, inducer strains, contain 10–15 copies of the *I* factor per haploid genome located in the euchromatic regions of the chromosome arms. These are not present in a few strains known as reactive strains. *I* factors transpose at low frequency in inducer strains but at high frequency in the female progeny of crosses between reactive and inducer flies. We have found that the activity of the *I* factor promoter is sensitive to the number of copies of the first 186 nucleotides of the *I* factor sequence, which constitutes the 5′-untranslated region. The activity of the *I* factor decreases as the copy number of this sequence increases.

Transposable elements are a substantial component of eukaryotic genomes, often making up >15% of the total DNA. There is probably strong selection for mechanisms that limit the frequency with which these elements transpose as transposition leads to an increase in the number of copies of an element and can cause insertional mutations and gross chromosome rearrangements. The mechanisms that restrict transposition are understood poorly, although they include element encoded regulatory molecules (1) and interactions between elements and the products of host genes (2). The *I* factor is a non-long terminal repeat, or LINE-like, retrotransposon (3). Like other elements of this type, it has no terminal repeats but possesses a deoxyadenosine-rich sequence at the 3′ end of one strand and has two ORFs, the first encoding a nucleic acid binding protein and the second a putative reverse transcriptase (3, 4). To transpose, the *I* factor is transcribed into a full length RNA that is reverse transcribed into a copy integrated at a new site in the genome (5–7).

All strains of *Drosophila melanogaster* studied so far contain 20–30 defective *I* factors per haploid genome located in pericentromeric DNA. The majority of strains, the so-called inducer strains, also contain 10–15 *I* factors in the euchromatic DNA of the chromosome arms. These include full length functional elements. A few strains lack these active elements and are known as reactive strains (8). The frequency of transposition of *I* factors in inducer strains is low but is increased by several orders of magnitude in the female progeny of crosses between reactive females and inducer males (9). This increase is not seen in the male progeny of such a cross and in females appears to be confined to the germ line, where it is associated with reduced fertility and an increased mutation rate. This phenomenon is called I-R hybrid dysgenesis, and the affected females are called SF females. The female progeny of reciprocal crosses, RSF females, appear normal, although the frequency of *I* factor transposition is increased in their germ-

line and is only approximately five times less than in SF females (9).

These observations suggest that *I* factors are subject to at least two forms of regulation, one that prevents transposition in inducer strains but permits transposition in the progeny of a dysgenic cross and the other that restricts transposition to the germ line of females. In each case, at least part of the regulation is exerted at the level of transcription since full length *I* factor transcripts can be detected by Northern blots only in RNA from the ovaries of SF and RSF females (5). The promoter that directs synthesis of this RNA is located within the first 30 bp of the *I* factor (10). A reporter gene linked to the first 186 nucleotides of the *I* factor, the 5′-untranslated region (5′-UTR), is expressed to a level that is ≈20-fold higher in ovaries than in nonovarian tissues because of an ovary specific enhancer between nucleotides 41 and 186 (11). This includes a sequence, called site 1, between position 138 and 157 that is recognized by a sequence-specific binding protein. Deletion of this sequence reduces the overall level of transcription from the *I* factor promoter and prevents the enhanced expression in ovaries, suggesting that it, and the protein or proteins that recognizes it, contribute to the activity of the enhancer (11).

The mechanism regulating *I* factor expression in inducer strains is much less well understood. It is the presence of *I* factors themselves that is responsible for this since a reactive strain can be converted to the inducer state simply by the introduction of a complete *I* factor (12, 13). The incoming element transposes in the germ line of females increasing in copy number as it does so. After a few generations, the number of copies reaches the level normally found in an inducer strain, by which point the frequency of transposition has dropped. This indicates that *I* factors regulate their own activity in inducer females and that their level of activity is sensitive to their copy number. Transcriptional control plays a part in this as expression of a reporter gene linked to the 5′-UTR of the *I* factor is reduced by ≈30-fold in the ovaries of inducer as compared with reactive females (11).

As no repressor of *I* factor transcription has been identified so far we suggest that increasing the number of copies of the *I* factor *per se* is responsible for this effect as has been seen for some transgenes in plants (14), *Drosophila* (15, 16) and mouse (17). The results reported here show that expression of the *I* factor promoter is reduced in the presence of increasing number of copies of the 5′-UTR indicating that this sequence can mediate a copy number effect on its own. This might be

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because the 5′-UTR binds a regulatory protein that is titrated until its level falls below that required to allow transposition or because multiple copies of the 5′-UTR lead to a reduction in expression due a change in chromatin structure as is proposed for cosuppression in plants (14) and *Drosophila* (15).

MATERIALS AND METHODS

Plasmid Construction. Plasmid pC42PIY was constructed by inserting the *Xba*I–*Bam*HI fragment of pI186 (10) into the *P* element transformation vector Carnegie 4 (18). A second copy of this sequence on a *Hinc*II–*Eco*RI fragment of pI186 then was inserted between the *Sma*I and *Eco*RI sites of the resulting plasmid. This gave plasmid pC42PI. A *Sal*I fragment containing the *yellow* gene (19) then was inserted at the *Sal*I site of pC42PI to give pC42PIY (Fig. 1). Plasmid pW83PI was made by first inserting the *Xba*I–*Eco*RI fragment of pI186 between the *Xba*I–*Eco*RI sites of pW8 (20) and then introducing the *Sma*I–*Pst*I fragment of pC42PIY containing two copies of the 5′-UTR between the *Hpa*I and *Pst*I sites of the resulting construct (Fig. 1). Plasmids pC42PIΔY and pW83PIΔ were constructed in the same way as pC42PIY and pW83PI but by using pIΔ186, from which site 1 (nucleotides 139–157) had been deleted (11), as the source of the 5′-UTR.

Construction of Transgenic *Drosophila* Stocks. All *P* element transformations (21) were carried out by using the reactive *yw* strain JA as the recipient. Insertions of C42PIY, which contains two copies of the 5′-UTR, and of C42PIΔY, which contains two copies of the deleted 5′-UTR, were selected by using the *yellow*$^+$ marker. Insertions C17 and CΔ9 are on chromosome II whereas insertions C30 and CΔ57 are on chromosome III. Insertions of W83PI, which contains three copies of the 5′-UTR, and of W83PIΔ, which contains three copies of the deleted 5′-UTR, were selected by using the *white*$^+$ marker. Insertions P22 and PΔ40 are on chromosome II whereas insertions P17 and PΔ20 are on chromosome III. Homozygous lines from the [y$^+$] transformants C17H, C30H, CΔ9H, and CΔ57H were established by using the reactive balancers *CyO* or *DcxF* whereas homozygous lines from the [w$^+$] transformants P22H, P17H, PΔ40H, and PΔ20H were established by selecting red-eyed flies from crosses between heterozygotes. The structure of the *P* element vectors inserted in each transgenic line was checked by Southern transfer experiments. To get strains with five copies of the 5′-UTR per haploid genome, C17H females were mated with P22H males, and C30H females were mated with P17H males. In each case, F$_1$ females were crossed with reactive *yw* males. Any [y$^+$w$^+$] progeny would have resulted from recombination between the transgenes, and these were used to construct the homozygous lines C17P22H and C30P17H.

The strain with 10 copies of the 5′-UTR per haploid genome was obtained from crosses between C17P22H and C30P17H and strains having appropriate reactive balancers. Females of strain C30P17H were mated with reactive *CyO* males and C17P22H females with reactive *DcxF* males. The [y$^+$w$^+$CyO] females resulting from the first cross were mated to [y$^+$w$^+$DcxF] males from the second cross. Red-eyed male and female progeny [y$^+$w$^+$CyODcxF] from this cross then were mated, and their [y$^+$w$^+$] sons and daughters, which did not carry balancers, were kept to establish the homozygous strain C17P22C30P17H.

Lines with a single functional *I* factor were made by using the *P* vector of plasmid pI954 (13), into which we had inserted a fragment of DNA containing the *yellow* gene. This was used to transform the reactive *yw* strain JA. Males of the G$_1$ generation that were [y$^+$] were crossed with JA females, and this was repeated at each subsequent generation. The transgenic male lines used in these experiments, 21.1 and 57.3, each contain a single copy of the full-length *I* factor on the second chromosome.

Chloramphenicol Acetyl Transferase (CAT) Assays of Extracts of *Drosophila* Ovaries. The activity of the *I* factor promoter was assayed by using strain 137, which contains a single insertion of the 186 nucleotides of the 5′-UTR linked to the coding sequence of the CAT gene from the bacterial transposon Tn9 followed by polyadenylation sequences from SV40. This was derived (10) from the plasmid pC4CAT (22). The CAT activity of ovarian extracts was measured as described in ref. 11. Extracts were made from 100 pairs of ovaries from 4- to 5-day-old females. Two assays were carried out for each ovary extract, and two extracts were prepared for females of each type tested.

Measurement of the Level of Sterility of SF Females. The level of sterility of SF females was measured by scoring the proportion of the eggs that did not hatch. In each experiment, at least two groups of 15 to 20 SF females were allowed to lay eggs overnight, 150–200 of which were left to develop at 25°C for 24 hours. All measurements were checked 1 or 2 days later.

Statistical Analysis. The relationship between the number of copies of the 5′-UTR and the levels of CAT activity or SF sterility were investigated by using Kendall's rank correlation analysis (23). This was done with the aid of the program STATVIEW STUDENT (Abacus Concepts, Berkeley, CA).

RESULTS

Increasing the Copy Number of the 5′-UTR. The region most likely to be involved in a copy number effect is the 186 nucleotides of the 5′-UTR because this contains sequences that reduce expression of the *I* factor promoter in inducer strains. We have investigated this by introducing varying number of copies of the 5′-UTR into a reactive strain by using *P* element transformation vectors. The vector C42PIY contains a copy of the *yellow* gene and two copies of the 5′-UTR whereas W83PI contains the *white* gene and three copies of the 5′-UTR. These then were used to transform the reactive strain JA, which carries mutations in the genes *white* and *yellow*.

Homozygous transformed lines with four or six copies of the 5′-UTR, that is, two or three copies per haploid genome, were established as indicated in Table 1. Strains homozygous for one copy of each of C42PIY and W83PI (five copies of the 5′-UTR per haploid genome) on either the second or third chromosome were constructed by isolating recombinants between
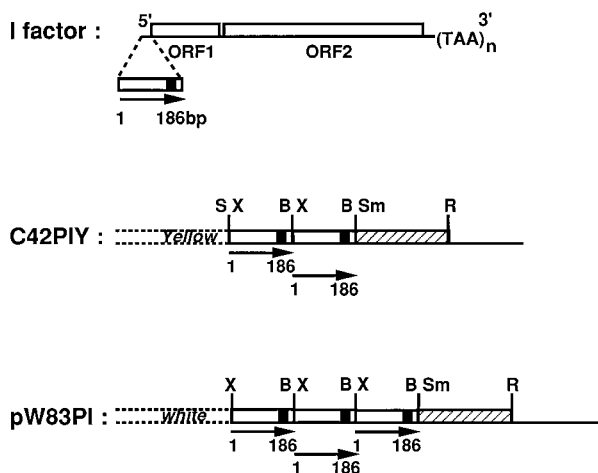

FIG. 1. Maps of the constructs C42PIY and pW83PI. The structure of the complete *I* factor showing the two ORFs (ORF1 and ORF2) is shown with the 5′-UTR expanded below. The position of site 1 is indicated in black. The regions of C42PIY and pW83PI containing the 5′-UTR are illustrated as well as adjacent regions of the *yellow* and *white* genes and the CAT reporter gene (striped box). The position of site 1 is indicated by the black box. B, *Bam*HI; R, *Eco*RI; S, *Sal*I; Sm, *Sma*I; X, *Xba*I.

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Table 1.   Transgenic lines carrying additional copies of the 5′-UTR of the *I* factor. The last column indicates the number of copies of the 5′-UTR *per* haploid genome

Vector	Line	Insertion site	Copies of 5′-UTR
C42PIY	C17H	Second	2
W83PI	P22H	Second	3
C42PIY	C30H	Third	2
W83PI	P17H	Third	3
(C42PIY +W83PI)	C17P22H	Second	5
(C42PIY +W83PI)	C30P17H	Third	5
2×(C42PIY +W83PI)	C17P22C30P17H	Second + Third	10
C42PIΔY	CΔ9H	Second	2
W83PIΔ	PΔ40H	Second	3
C42PIΔY	CΔ57H	Third	2
W83PIΔ	PΔ20H	Third	3
(C42PIΔY +W83PIΔ)	CΔ9PΔ40H	Second	5
(C42PIΔY +W83PIΔ)	CΔ57PΔ20H	Third	5

single insertions on these chromosomes as indicated in Table 1 and in *Materials and Methods*. Finally, a strain carrying 10 copies of the 5′-UTR per haploid genome was generated by combining recombinant second and third chromosomes (see *Materials and Methods*). These strains have been used to investigate the effect of increasing the number of copies of the 5′-UTR by measuring their effect on expression of a reporter gene linked to the *I* factor promoter and by measuring their effect on the fertility of SF females.

Effect on Expression of the I Factor Promoter. We have used a CAT reporter gene (*Materials and Methods*) to measure directly the activity of the *I* factor promoter. Females of strains with increasing copies of the 5′-UTR were mated with males of strain 137 (10), which has a single insertion of the 5′-UTR linked to the CAT gene, and CAT expression was measured in the ovaries of their female progeny. This allowed us to measure CAT expression in the presence of 1, 3, 4, 6, or 11 copies of the 5′-UTR per haploid genome. As the number of copies of the 5′-UTR increases, the level of CAT expression falls (Fig. 2). This is reproducible because similar results were obtained with two series of strains, those with insertions of 5′-UTR on the second chromosome (insertions C17 and P22) and those with insertions on the third (insertions C30 and P17).
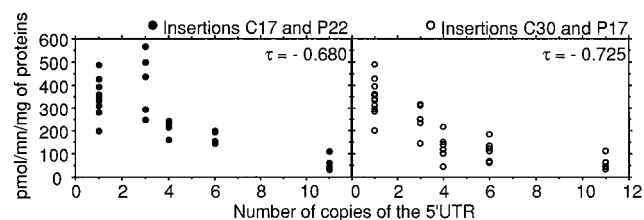

FIG. 2.   The effect of increasing doses of the 5′-UTR on the activity of the *I* factor promoter. The vertical axis indicates the CAT activity (pmol/min/mg of protein) in extracts of ovaries from females obtained as indicated below. The horizontal axis indicates the number of copies of the 5′-UTR per haploid genome. Females of strains with 0, 2, 3, 5, or 10 copies of the 5′-UTR per haploid genome were crossed with males carrying the 1–186:CAT reporter gene. This gave progeny with 1, 3, 4, 6, or 11 copies of the 5′-UTR per haploid genome. The black circles indicate the results of crosses using females of strains C17H, P22H, and C17P22H whereas the white circles indicate the results for strains C30H, P17H, and C30P17H. The final point (11 copies) was obtained by using females of strain C17P22C30P17H (Table 1).

We have used Kendall's rank correlation method (23) to analyze the relationship between CAT activity and the number of copies of the 5′-UTR. This shows that there is a significant negative correlation between CAT activity and 5′-UTR copy number in both experiments. The values of Kendall's τ are significantly negative both for flies derived from lines carrying insertions C17 and P22 ($\tau = -0.680$, $P = 0.0001$) and flies derived from lines carrying insertions C30 and P17 ($\tau = -0.725$, $P = 0.0001$). Furthermore, there is a linear relationship (not shown) between the reciprocal values of CAT activity and 5′-UTR copy number for both experiments, supporting our conclusion that the activity of the *I* factor promoter is related inversely to number of copies of the 5′-UTR.

Effect on the Fertility of I-R Dysgenic Females. SF females have lowered fertility because a proportion of their eggs do not hatch. This is also a measure of *I* factor expression, albeit an indirect one, as the proportion of eggs that do not hatch is correlated positively with the frequency of *I* factor transposition (24). In view of this, we have measured the fertility of dysgenic females resulting from crosses between inducer males carrying a single functional *I* factor and females of our reactive strains with increasing doses of the 5′-UTR. If males from a wild-type inducer strain were used in this experiment, the progeny would contain a defined number of copies of the 5′-UTR from their mothers but an unknown number from their fathers. We have, therefore, constructed lines of *D. melanogaster*, lines 21.1 and 57.3, in which males have a single functional *I* factor. This is possible since *I* factors do not transpose in males (9).

The sterility of SF females produced in this way (the proportion of their eggs that did not hatch) decreased as the number of copies of the 5′-UTR increased, indicating that the activity of the *I* factor also had decreased. The relationship between the proportion of eggs that did not hatch and the number of copies of the 5′-UTR is shown in Fig. 3. We again have analyzed the data by using Kendall's rank correlation. In each case, the values of Kendall's τ are significantly negative, as indicated in the figure legend. This is seen most clearly for females coming from crosses with males derived from line 57.3. In this case, the proportion of eggs that did not hatch fell from ≈95% for females with one copy of the 5′-UTR to <20% for those with 11 copies (Fig. 3*A*). Females from crosses with males derived from line 21.1 were less fertile than the corresponding females from crosses with line 57.3 (Fig. 3*B*, 1), possibly because the position of the *I* factor present in line 21.1 allows a higher level of expression. Since the fertility of SF females increases as they get older (25, 26) females from crosses with males from strain 21.1 were allowed to age for a further 2 weeks, and the hatching of their eggs was scored again. The proportion of eggs that did not hatch decreased for each class of SF females and was again higher in the presence of higher doses of the 5′-UTR (Fig. 3*B*, 2).

Site 1 Is Not Essential for the Copy Number Effect. Because at least some of the sequences required for normal expression of the *I* factor are located within the 5′-UTR (10, 11) increasing its copy number might titrate one or more regulatory factors, such as the factor binding to site 1 (11). We have tested whether site 1 is required for the copy number effect described above by repeating these experiments but using the 5′-UTR sequence with site 1 deleted. Homozygous lines again were established for insertions on the second and third chromosomes, and these were combined to give strains with 2, 3, or 5 copies of the deleted 5′-UTR per haploid genome (Table 1).

We have measured the effect of the deleted 5′-UTR on the activity of the *I* factor promoter by crossing these lines with strain 137 carrying the 1–186 sequence linked to the CAT gene. The CAT activity in the ovaries of the female progeny decreased significantly as the number of copies of the deleted 5′-UTR increased (Fig. 4), giving values for Kendall's τ of -0.578 ($P = 0.0018$) for crosses involving insertions CΔ9 and PΔ40 and

A - Paternal strain 57.3 :

B - Paternal strain 21.1 :
1 - Maternal strains C17H, P22H, C17P22H, C17P22C30P17H :

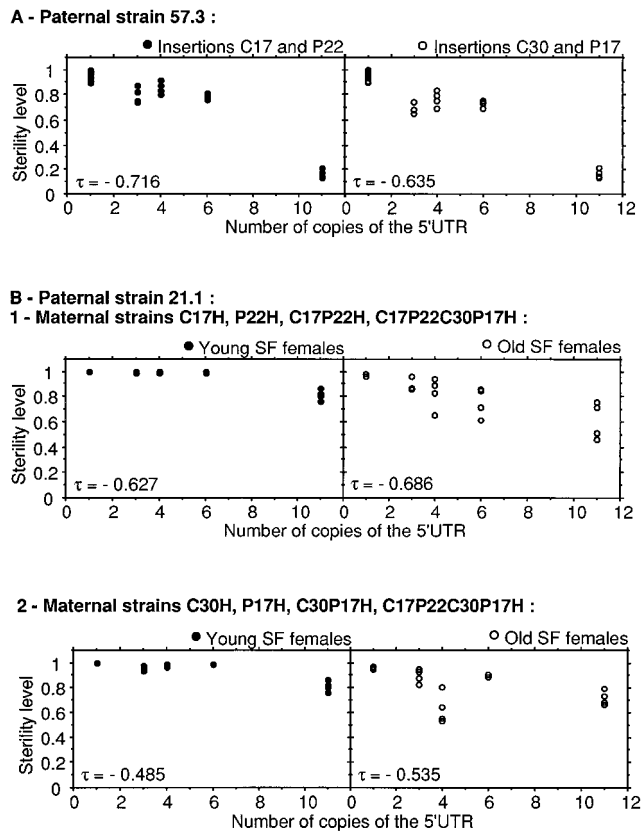**2 - Maternal strains C30H, P17H, C30P17H, C17P22C30P17H :**

FIG. 3.   The effect of increasing doses of the 5′-UTR on the sterility of SF females. The vertical axis indicates the proportion of the eggs laid by SF females that did not hatch whereas the horizontal axis indicates the number of copies of the 5′-UTR per haploid genome. The SF females were produced by crossing males with a single active *I* factor with females of strains with 0, 2, 3, 5, or 10 copies per haploid genome as follows. Males with a single *I* factor were the sons of crosses between females of a reactive strain marked with *y, w;CyO* and males of the lines 21.1 or 57.3 carrying single *I* factors located on the second chromosome and linked to y^+. Females of lines with varying numbers of copies of the 5′-UTR then were mated with the [y^+; CyO] male progeny of this cross. (*A*) Proportion of unhatched eggs laid by 3- to 4-day-old SF females from crosses using males from strain 57.3. The black circles indicate the results for SF females derived from females of strains C17H, P22H, and C17P22H whereas the white circles indicate the results for SF females derived from females of strains C30H, P17H, and C30P17H. The final point (11 copies) was obtained by using females of strain C17P22C30P17H (Table 1). Kendall's τ corrected for ties are -0.716 ($P = 0.0001$) and -0.635 ($P = 0.0001$), respectively. (*B*) Proportion of unhatched eggs laid by 3- to 4-day-old SF females derived from crosses using males from strain 21.1. The black circles are for eggs laid by 3- to 4-day-old females whereas the white circles are for eggs laid by 16- to 20-day-old females. (1) SF females derived from females of strains C17H, P22H, C17P22H, and C17P22C30P17H. Kendall's τ corrected for ties are -0.627 ($P = 0.0003$) and -0.686 ($P = 0.0001$), respectively. (2) SF females derived from females of strains C30H, P17H, C30P17H, and C17P22C30P17H. Kendall's τ corrected for ties are -0.485 ($P = 0.0028$) and -0.535 ($P = 0.0014$), respectively.

-0.596 ($P = 0.0013$) for crosses involving insertions CΔ57 and PΔ20. The fertility of SF females also was affected by the deleted 5′-UTR. When females carrying these insertions were mated with males carrying a single functional *I* factor, the proportion of eggs laid by their daughters that did not hatch decreased as the number of copies of the deleted 5′-UTR increased (data not shown), although this effect was less marked than observed previously with the wild-type 5′-UTR. For 3- to 4-day-old SF females from crosses with males carrying the single *I* factor insertion 57.3, the values of Kendall's τ was -0.298 ($P = 0.107$) for SF females with 0–5
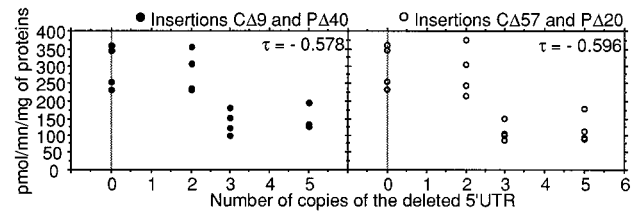

FIG. 4.   The effect of increasing doses of the 5′-UTR with site 1 deleted on the activity of the *I* factor promoter. The vertical axis indicates the CAT activity (pmol/min/mg of protein) in ovarian extracts from females obtained as indicated below. The horizontal axis indicates the number of copies of the deleted 5′-UTR per haploid genome. Females of strains with 0, 2, 3, or 5 copies of the deleted 5′-UTR per haploid genome were crossed with males carrying the 1–186:CAT reporter gene. This gave progeny with 0, 2, 3, or 5 copies of the deleted 5′-UTR per haploid genome. The black circles indicate the results of crosses using females of strains CΔ9H, PΔ40H, and CΔ9PΔ40H whereas the white circles indicate the results for strains CΔ57H, PΔ20H, and CΔ57PΔ20H (Table 1).

copies of the deleted 5′-UTR using insertions CΔ9 and PΔ40 and was -0.723 ($P = 0.0001$) for crosses involving insertions CΔ57 and PΔ20. For crosses with males carrying the single *I* factor insertion 21.1, a significant effect could only be seen when scoring eggs laid by SF females carrying 0–5 copies of the insertions CΔ57 and PΔ20. The limited effect seen with insertions CΔ9 and PΔ40 may be caused by the DNA flanking one or other of these insertions. These results suggest that deleting site 1 reduces but does not abolish the effect of the 5′-UTR on *I* factor expression.

DISCUSSION

The reduced expression of the *I* factor that we have found associated with increasing dosage of the 5′-UTR is similar to the behavior of a complete *I* factor introduced into a reactive strain. Initially, the *I* factor transposes at high frequency, but this drops as its copy number increases, falling to a low level when this reaches ≈15 copies per haploid genome (12, 13). The number of copies of the 5′-UTR is probably less than this as *I* factors often are truncated from the 5′ end during transposition (6, 27, 28).

The copy number effect we have observed is unlikely to be caused by a protein encoded by the 186 bp of the 5′-UTR as it does not contain a methionine codon. An RNA is also unlikely to be involved as deletion of site 1 reduces transcription from the *I* factor promoter ≈100-fold (11) but does not prevent the 5′-UTR from affecting *I* factor expression (Fig. 4). This suggests that a mechanism similar to quelling in *Neurospora crassa* (29) is not involved, although this cannot be ruled out entirely at this stage.

If the copy number effect cannot be attributed to an RNA or protein, then it may be caused by the DNA itself, perhaps because increasing copies of the 5′-UTR titrate a factor required for *I* factor expression or because they stimulate a change in chromatin structure that reduces *I* factor transcription. Activation of *fushi tarazu* during *Drosophila* embryogenesis may be explained by titration of the *tramtrack* protein, which inhibits its transcription (30). *Fushi tarazu* is activated as a particular nucleocytoplasmic density is reached in the developing embryo. Expression can be advanced or retarded by decreasing or increasing the level of tramtrack protein, suggesting that titration of tramtrack with increasing density of nuclei in the cytoplasm allows transcription to start at a particular stage of embryogenesis. The protein, or proteins, that we have identified as binding to site 1 (11) are probably not candidates for such a titratable transcription factor as deletion of site 1 does not abolish the copy number effect, although it is reduced somewhat. Either a protein binding to

some other region of the 5′-UTR is being titrated or another mechanism is responsible for this effect.

Dorer *et al.* (31) have found that expression of a mini-*white* gene is reduced when it is present in tandemly repeated copies of a *P* element transformation vector. This phenomenon has been called "repeat-induced gene silencing," and a similar effect has been reported for tandemly repeated genes in the mouse (17). In *Drosophila*, the inactivated gene behaves as if it is in heterochromatin and subject to position effect variegation (32, 33) because the inactivation can spread to adjacent chromosomal sequences. It also can spread between copies of the transgene in different parts of the genome, but there is no evidence that it affects the expression of the endogenous *white* gene. Although the *P* element transformation vectors that we have used in these experiments contain two or three copies of the 5′-UTR repeated in tandem, we have observed no consistent variegation in expression of the *white* or *yellow* genes that were used as transformation markers in these experiments, even in flies with 11 copies of the 5′-UTR per haploid genome. This suggests that any heterochromatinization of the 5′-UTR does not spread far from the tandemly repeated *I* factor sequences, even though it was able to affect a single copy of the *I* factor promoter linked to the CAT reporter gene or a single complete *I* factor elsewhere in the genome.

The presence of transgenes can lead to a reduction in the expression of homologous genes in plant cells in a phenomenon known as "cosuppression" (14) and a possible example of cosuppression has been described recently in *Drosophila*. Expression of the *alcohol dehydrogenase* gene, *adh*, was found to be reduced in the presence of increasing numbers of copies of a hybrid transgene comprising the promoter and regulatory sequences of the *white* gene fused to the coding region of *adh*. (15). Expression of both of the hybrid and endogenous *adh* genes decreased as the number of copies of the transgene increased from one to six copies per cell. This appears to be the result of an altered state of the chromatin in the vicinity of the normal and hybrid *adh* genes because expression was increased by mutations in the *Polycomb*-group genes *Polycomb* and *Polycomb-like*, which code for proteins required to keep genes in an inactive state during development. We shall investigate whether or not *Pc* mutations reduce the copy number effect that we have observed and shall look for an association of Polycomb protein and the *I* factor promoter in the presence of multiple copies of the 5′-UTR.

Several authors have suggested that phenomena that reduce the expression of repeated sequences may have evolved to protect genomes from invasion by transposable elements (14, 15), and this may be the origin of the copy number effect that we have seen for the 5′-UTR of the *I* factor. Copy number control of *I* factor activity can explain why transposition is induced in crosses between reactive and inducer strains as the number of copies of the 5′-UTR in the progeny of such a cross will be one-half that in the inducer parent, and any inhibition caused by the number of copies of the 5′-UTR would be reduced until this was restored to the inducer level by transposition. This mechanism would benefit the host as it would keep the *I* factor in check, and it would benefit the *I* factor itself because it would ensure that its copy number increased by transposition if it should fall below the threshold level. Of interest, Cavalli and Paro recently have found that a state of gene activity caused by an adjacent polycomb response element is transmitted through the female germ line but not the male (34). This could explain why I-R hybrid dysgenesis is seen in the progeny of crosses between reactive females and inducer males but not in the progeny of the reciprocal cross.

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