New Insights on Homology-Dependent Silencing of *I* Factor Activity by Transgenes Containing ORF1 in *Drosophila melanogaster*

Sophie Malinsky, Alain Bucheton and Isabelle Busseau

Institut de Génétique Humaine, CNRS, 34396 Montpellier Cedex 05, France

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ABSTRACT

I factors in *Drosophila melanogaster* are non-LTR retrotransposons that transpose at very high frequencies in the germ line of females resulting from crosses between reactive females (devoid of active *I* factors) and inducer males (containing active *I* factors). Constructs containing *I* factor ORF1 under the control of the *hsp70* promoter repress *I* factor activity. This repressor effect is maternally transmitted and increases with the transgene copy number. It is irrespective of either frame integrity or transcriptional orientation of ORF1, suggesting the involvement of a homology-dependent *trans*-silencing mechanism. A promoterless transgene displays no repression. The effect of constructs in which ORF1 is controlled by the *hsp70* promoter does not depend upon heat-shock treatments. No effect of ORF1 is detected when it is controlled by the *I* factor promoter. We discuss the relevance of the described regulation to the repression of *I* factors in I strains.

I factors in *Drosophila melanogaster* are non-long terminal repeat (LTR) retrotransposons of particular interest because high frequencies of transposition can be induced experimentally, resulting in the phenomenon of IR hybrid dysgenesis (Bucheton 1990; Busseau *et al.* 1994). All *D. melanogaster* strains fall into one of two categories: inducer (I) strains, which contain active *I* factors, and reactive (R) strains, which do not. *I* factors contain two open reading frames (ORFs): ORF1, which encodes an unknown function, and ORF2, which putatively encodes a polypeptide-containing endonuclease, reverse transcriptase, and ribonuclease H domains (Fawcett *et al.* 1986; Abad *et al.* 1989). *I* factors are stable in inducer strains but transpose very efficiently in the germ line of hybrid females resulting from crosses between the two classes of strains. The highest levels of retrotransposition are observed in females, denoted SF (for Stérilité Femelle), that are produced by crosses between females from a reactive strain and males from an inducer strain. Transposition also occurs in females, denoted RSF (for Réciproque Stérilité Femelle), that are produced by reciprocal crosses between females from an inducer strain and males from a reactive strain, but at frequencies that are lower than in SF females. Transposition is restricted to the female germ line. Accumulation of data reported by several authors (Chaboissier *et al.* 1990; Lachaume *et al.* 1992; Lachaume and Pinon 1993; McLean *et al.* 1993) indicate that *I* factor activity depends primarily on specific transcription initiated from an internal promoter localized entirely within the *I* factor 186-bp 5′ untranslated region (UTR). This promoter is repressed in inducer strains but activated when introduced in the germ line of reactive strains, and it drives the synthesis of a full-length transcript that is believed to act as both the retrotransposition intermediate and the messenger for translation of the products of ORF1 and ORF2 (Chaboissier *et al.* 1990; Bouhidel *et al.* 1994). The abundance of transcripts at the time of transposition appears correlated with the transposition frequency (Chaboissier *et al.* 1990; McLean *et al.* 1993).

In addition to high levels of transposition occurring in their germ line, SF females display a characteristic syndrome of sterility: a proportion of the eggs they lay fail to hatch and embryos die during early development. RSF females are normally fertile. Interestingly, the intensity of SF sterility, *i.e.*, the proportion of eggs that do not hatch, appears correlated to some extent with the frequency of *I* factor retrotransposition, although the relationship between transposition and sterility is unclear (Picard 1978). Therefore, the percentage of eggs that do not hatch is a rough estimate of the intensity of *I* factor activity in the germ line of SF females, within a range of observation between boundaries defined by normal fertility and total sterility corresponding to thresholds of detection and saturation, respectively.

I factors clearly self-regulate their activity since retrotransposition rarely occurs in inducer strains. When a single *I* factor is introduced into the genome of a reactive line, it transposes during a few generations until the number of copies reaches a certain point, estimated to be ~10–15, above which the strain becomes inducer and transposition stops (Picard 1978; Pritchard *et al.* 1988). This self-regulation has a maternal component,

Corresponding author: Isabelle Busseau, IGH-CNRS, 141 rue de la Cardonille, 34396 Montpellier Cedex 05, France.
E-mail: busseau@igh.cnrs.fr

as is evidenced by the fact that the two reciprocal crosses are not equivalent: retrotransposition is less efficient in RSF females, having received *I* factors from their mother, than in SF females, having received *I* factors from their father.

Recent results have shed some light on the question of how *I* factors self-regulate. Chaboissier *et al.* (1998) showed that *I* factor activity decreases in the presence of several copies of the 5′ UTR in the genome and that this effect correlates with a decrease of transcriptional activity of the *I* factor promoter. Jensen *et al.* (1999a,b) showed that some other parts of the *I* factor (ORF1 and a small part of ORF2) have a repressor effect on *I* factor activity when they are under the control of the *hsp70* promoter. This repressor effect is independent from the translation of a protein product, it increases with the copy number and over generations, and it is maternally transmitted. These observations suggest that self-regulation of the *I* factor is mediated by homology-dependent *trans*-silencing.

In this article we report independent results that confirm the results of Jensen *et al.* (1999a,b) and bring some new insights on homology-dependent silencing of *I* factor activity by ORF1-containing transgenes.

MATERIALS AND METHODS

Fly stocks: All strains used in the experiments are M in the PM system of hybrid dysgenesis (Engels 1989). The genetic nomenclature follows (Lindsley and Zimm 1992). w^{1118} is an inducer strain. The strong reactive *wK* strain was obtained from Lüning (1981). The strong reactive strain *JA* carries *y* and *w* mutations.

Plasmid constructions and transgenic lines: Basic molecular biology techniques were adapted from Sambrook *et al.* (1989). All cloned sequences from the *I* factor derive from pI407 (Bucheton *et al.* 1984), which contains an active *I* factor (Pelisson 1981; Pritchard *et al.* 1988). Site positions are given according to the sequence published by Fawcett *et al.* (1986). Construction of hsORF2 was described previously and is referred to as phsORF2-HN in Busseau *et al.* (1998). hsORF1 and hsORF1as (Figure 1) were obtained by ligation of the *Hpa*II-Klenow-treated *Hpa*I fragment that contains all ORF1 (positions 161–1492) from pI407 into the *Hpa*I site of the pCaSpeR-*hs* vector (Thummel and Pirrotta 1991) and of the pCaSpeR-*hs* vector deleted of the *Bam*HI fragment containing the *hsp70* 3′ UTR, respectively. hsORF1fs is identical to hsORF1 except that a frameshift was introduced by insertion, using PCR with relevant oligonucleotides, of a T at position 191, just after the first ATG of ORF1, leading to the substitution of the sequence ATGACAGA . . . by ATGATCAGA . . . pI, ORF1, and pIORF1 were constructed in two steps.

pI: the 5′ UTR of the *I* factor was extracted from pI186 (McLean *et al.* 1993) as a *Hin*dIII-*Bam*HI fragment and ligated to the same sites in the polylinker of pBluescript-KS$^-$, producing the pBT186 plasmid; then the 5′ UTR was extracted from pBTI186 as a *Kpn*I-*Spe*I fragment that was ligated to the *Kpn*I and *Spe*I sites of pCaSpeR-4 vector.

ORF1: the *I* factor ORF1 sequences were extracted from pI407 as a *Msp*I-*Eco*RV fragment and ligated to the same sites in the polylinker of pBluescript-KS$^-$, producing the pBTORF1 plasmid; then the ORF1 sequences were extracted from pBTORF1 as an *Eco*RI-*Hpa*I fragment that was ligated to the *Eco*RI and *Stu*I sites of the pCaSpeR-4 vector (Thummel and Pirrotta 1991).

pIORF1: the 5′ UTR + ORF1 sequences of *I* were extracted from pI407 as a *Cla*I-*Hin*dIII fragment and ligated into the same sites in the polylinker of pBluescript-KS$^-$, producing the pBTIORF1 plasmid; then the 5′ UTR + ORF1 sequences were extracted from pBTIORF1 as an *Eco*RI-*Hpa*I fragment that was ligated to the *Eco*RI and *Stu*I sites of the pCaSpeR-4 vector.

P-element-based transformations were essentially as described by Spradling and Rubin (1982), except that pUC *hs*pΔ2-3 (Flybase ID no. FBmc0002087) was coinjected as the source of transposase. The recipient strains were *wK* or *JA*. Several independent homozygous transgenic lines were established for each construct by selecting dark orange-eyed flies, chromosome localizations of the transgenes were determined using balancer stocks, and integrity of the transgenes was checked by Southern blot analyses.

Crosses and measurements of female fertility: All crosses were performed on standard fly medium (Gans *et al.* 1975) at 23° except where otherwise stated. Typically samples of 10–13 virgin females were mated to 8–10 males. They were transferred on fresh medium when necessary, until they were 8–10 days old, and then they were discarded. To determine the intensity of female sterility, 8–13 young SF females (<3 days old) were mated with the same number of males (brothers), transferred onto fresh medium the next day, and discarded the day after. Eggs were put to develop 24 hr at 25° and the percentage of hatched eggs was determined. Only measures of samples of >100 embryos were taken into account. Heat-shock treatments were applied during two successive generations by placing all developmental stages (from embryos to egg-laying adults) at 37° 1 hr once a day.

RESULTS

The repressor effect of ORF1 sequences is independent from the amount of ORF1 transcript: Constructs in which ORF1 and ORF2 are controlled by the *D. melanogaster hsp70* heat-inducible promoter (Figure 1) were introduced into the genome of the reactive strain *wK*. Homozygous transgenic lines *HH* and *HN*, containing the transgenes *hsORF1* and *hsORF2*, respectively, (Figure 1) were established and maintained over 2 years (∼35 generations) at 20° before experiments. Each transgenic line contains one homozygous copy of the transgene, except *HH9*, which contains two. Each transgenic line was then divided into two sublines (including the recipient strain *wK*), and one of the sublines was submitted every day to a heat treatment of 1 hr at 37°. We verified that the heat treatment had no effect *per se* on the fertility of females from the *wK* strain (data not shown). We also checked by Northern blot analyses that they resulted in a large amount of ORF1 or ORF2 transcripts among the RNAs extracted from whole flies, whereas these transcripts are not detectable in the absence of heat treatments (Figure 2). In addition it has to be noted that *HN* transgenic lines were used in another study to show that *hsORF2* transgenes can mobilize the defective *I* element *IviP2* (Busseau *et al.* 1998). *Trans*-complementation was more efficient upon heat induction, indicating that the *hsp70* promoter is heat inducible at the time of *I* factor transposition.

Transgenic lines In *wK*	Transgenic lines In *JA*	Repressor effect
HN	-	no
HH	*HOU*	yes
-	*HOUf*	yes
-	*HOUas*	yes
-	*H*	no
-	*OU*	no
-	*pIOU*	no
-	*pI*	no

FIGURE 1.—Constructs and transgenic lines. Drawings represent the *I* factor (top) and the different transgenes used in this study. ORF1 and ORF2 are indicated as open boxes while untranslated regions from the *I* factor (5′I and 3′I, 5′ and 3′ untranslated regions, respectively) are indicated as solid boxes. Light strippled boxes indicate the promoter (phs) and the terminator region (ths) of the *hsp70* gene, while heavy strippled boxes indicate the 3′ end of the *P* element (3′P). Arrows indicate the position of transcription initiation from the *I* factor or the *hsp70* promoter. Parts of the transgenes that are irrelevant to the study (*white* sequences and the 5′ end of the *P* element) are omitted. On the left of the drawings are the names of the different transgenes. On the right of the drawings are the names of the transgenic lines derived from the *wK* and *JA* strains, and the last column summarizes the repressor effects of the transgenes on *I* factor activity.

After two generations, the fertility of SF females issued from crosses between females from transgenic lines and males from the w^{1118} inducer stock was measured (Figure 3) by determining the hatching percentage of their eggs. SF females issued from the strongly reactive *wK* control were, as expected, strongly sterile, with a percentage of hatching eggs close to 0% when they were <3 days old (Figure 3a) and increasing as they got older (6 and 12 days old, Figure 3b). This hatching percentage was not affected when the *wK* strain was submitted to a daily heat treatment for two generations before the dysgenic cross (Figure 3, a and b). This indicates that a daily heat shock at 37° for 1 hr in our experimental conditions has no observable effects on reactivity levels, contrary to long thermal treatments at 29° (BUCHETON 1978, 1979a,b). By contrast, females of *HH* lines produced SF females that were less sterile, with the hatching percentages of the eggs laid by these SF females varying between 10 and >60% (Figure 3a). SF females issued from females of the sublines submitted to heat shocks every day did not display fertility levels different from those of SF females derived from females of the sublines that did not receive heat-shock treatments. This indicates that *hsORF1* transgenes, containing the *I* factor ORF1 under the control of the *hsp70* promoter, have a repressor effect on *I* factor activity and that this effect is independent from heat induction of the *hsp70* promoter. By contrast, *hsORF2* transgenes have no repressor effect on *I* factor activity: *HN* transgenic lines were also tested ~100 (*HN9* and *HN27*) and 140 (*HN27*) generations after transgenesis and still produced highly sterile SF females, whereas *HH* lines reproducibly produced SF females that were less sterile than SF females from the control cross (data not shown). All transgenic lines contain one homozygous copy of the transgene, except *HH9*, which contains two, and they produce the less sterile SF females.

We tested the repressor effect of *hsORF1* transgenes in another genetic background. Transgenic lines *HOU*, containing the same *hsORF1* transgene as *HH* lines, and transgenic lines *HOUf*, containing the *hsORF1fs* transgene in which a frameshift just after the first ATG prevents expression of the ORF1 protein (Figure 1), were established by *P*-mediated transformation of the reactive strain *JA*. The establishment of each transgenic line was accompanied by the parallel establishment of a sibling nontransgenic line as a control. The transgenic and nontransgenic lines were derived, respectively, from orange- and white-eyed individuals issued from the same transformed fly. Dysgenic crosses of females of the lines with males from the w^{1118} inducer stock were performed and the fertility of the resulting SF females was measured. Data are shown in Figure 4. *HOU* and *HOUf* transgenic lines produced SF females that were less sterile than nontransgenic females from the internal control. The fertility improvements appear weak a few generations after transgenesis (*HOU* at generation 6, *HOUf* at generation 8) but are more obvious at generations 40–42, except for line *HOU57a*, which shows no effect. It is noticeable that two transgenic lines, *HOU33* and *HOUf29.3*, that produce the less sterile SF females, contain two homozygous copies of the transgenes, whereas other transgenic lines all have only one copy. These data indicate first that the repressor effect of *hsORF1* transgenes is also effective in the *JA* background and second that *hsORF1* transgenes can repress *I* factor activity independently from expression of an ORF1 protein. They also suggest that two copies of a transgene may have a stronger repressor effect than one copy.

The repressor effects of two *hsORF1* transgenes are

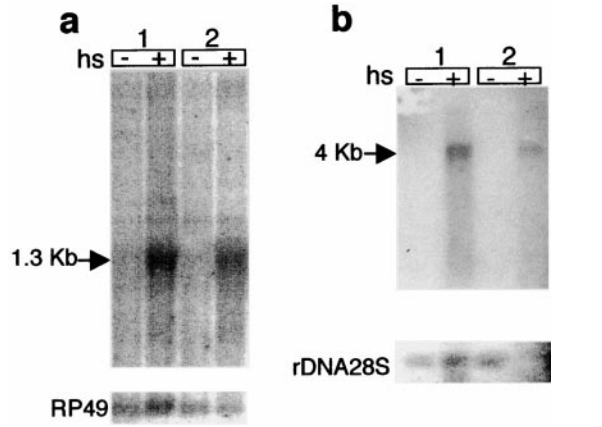

FIGURE 2.—Analyses of ORF1-containing RNAs from *HH* and *HN* transgenic lines. (a) Northern blots of total RNAs extracted from transgenic lines HH9 (1) and HH17 (2) submitted (+) or not (−) to heat shock, hybridized with an RNA probe containing ORF1. Below is shown the result of hybridization of the same membrane with an RP49 probe as a control of RNA amounts. (b) Northern blots of total RNAs extracted from transgenic lines HN45 (1) and HN27 (2) submitted (+) or not (−) to heat shock, hybridized with an RNA probe containing ORF2. Below is shown the result of hybridizing the same membrane with an rDNA28S probe as a control of RNA amounts.

cumulative: We tested whether the regulatory effect of *hsORF1* is enhanced by increasing the number of copies of the transgene. Using lines *HH13* and *HH16* (transgenes on chromosomes *II* and *III*, respectively), we constructed various sublines as shown in Figure 5a. Reciprocal crosses between flies from lines *HH13* and *HH16* were made to obtain hybrid females heterozygous for both transgenes. These hybrid females were crossed to males of the *wK* strain. The progeny of these crosses were scored upon eye color intensity and mated to establish sublines. *1613-1*, *1613-2*, *1613-3*, *1613-4*, and *1613-5*, containing both transgenes at the homozygous state, *1613-16H* and *1613-13H*, containing one homozygous transgene on chromosomes *III* and *II*, respectively, and *1613-wK*, devoid of transgene, were obtained in the progeny of the crosses between *HH16* females and *HH13* males. *1316-7* and *1316-8*, containing both transgenes at the homozygous state, and *1316-13H*, containing one homozygous transgene on chromosome *II*, were obtained in the progeny of the reciprocal cross between *HH13* females and *HH16* males. The fertility of SF females obtained from crosses between females of these sublines and males of the w^{1118} inducer stock was measured three generations after establishment of the sublines. As shown in Figure 5b, sublines *1613* and *1316*, containing two homozygous transgenes, reproducibly produce less sterile SF females than sublines containing only one homozygous transgene, whereas the *1613-wK* subline, devoid of transgene, produces strongly sterile SF females, as does the strongly reactive *wK* strain. Subsequent tests made 29 generations after establishment produced similar results (not shown). Therefore, the effect of *hsORF1* transgenes is cumulative.

The repressor effect of *hsORF1* transgenes requires the association of the *hsp70* promoter and ORF1 sequences: We have tested the ability of the *hsp70* promoter and of ORF1 sequences without any promoter to repress *I* factor activity. For this, transgenic lines *H*, containing the pCaSpeR-*hs* vector, and *OU*, containing the sequences of ORF1 (see Figure 1), were established by *P*-mediated transformation of the *JA* strain. They all contain one homozygous copy of the transgene. Nontransgenic sibling lines were also established as controls as described above and the regulatory effects of the transgenes were measured at generations 5, 8, 9, 12, and 13 after transgenesis in the case of *OU* lines and at generations 4 and 16 in the case of *H* lines. Typical data are shown in Figure 4: none of the transgenes exhibit a regulatory effect. This indicates that the regulatory effect of the *hsORF1* transgenes requires the association of the sequences of the *hsp70* promoter and of ORF1.

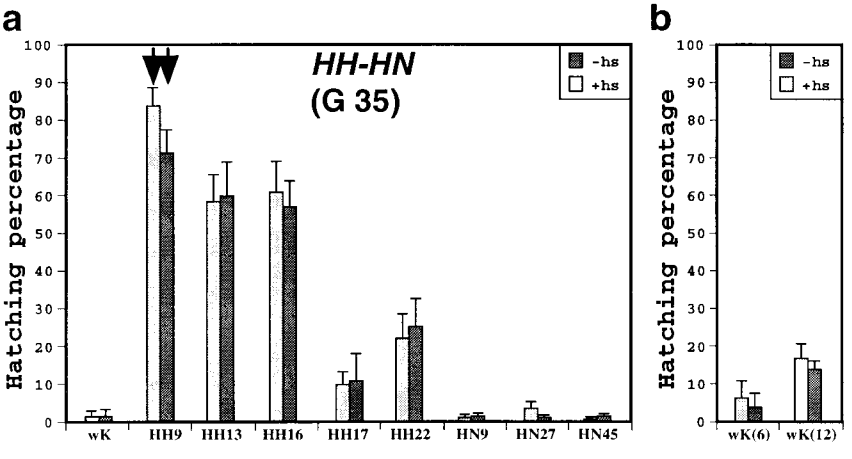

FIGURE 3.—Repressor effect of *hsORF1* and *hsORF2* transgenes. (a) Hatching percentages of the eggs laid by SF females issued from crosses between females from the *HH* and *HN* transgenic lines obtained from the reactive stock *wK*, raised at 20°, and submitted or not every day to a heat treatment during two successive generations, 2 yr (~35 generations, except *HH9*, for which the experiment was done three generations later) after transgenesis, and males from the w^{1118} inducer stock. Arrowheads point to transgenic line *HH9* containing two homozygous transgenes. (b) Hatching percentages of the eggs laid by 6-day-old [*wK*(6)] and 12-day-old [*wK*(12)] SF females issued from crosses between females from the reactive stock *wK* submitted or not every day to a heat treatment and males from the w^{1118} inducer stock.

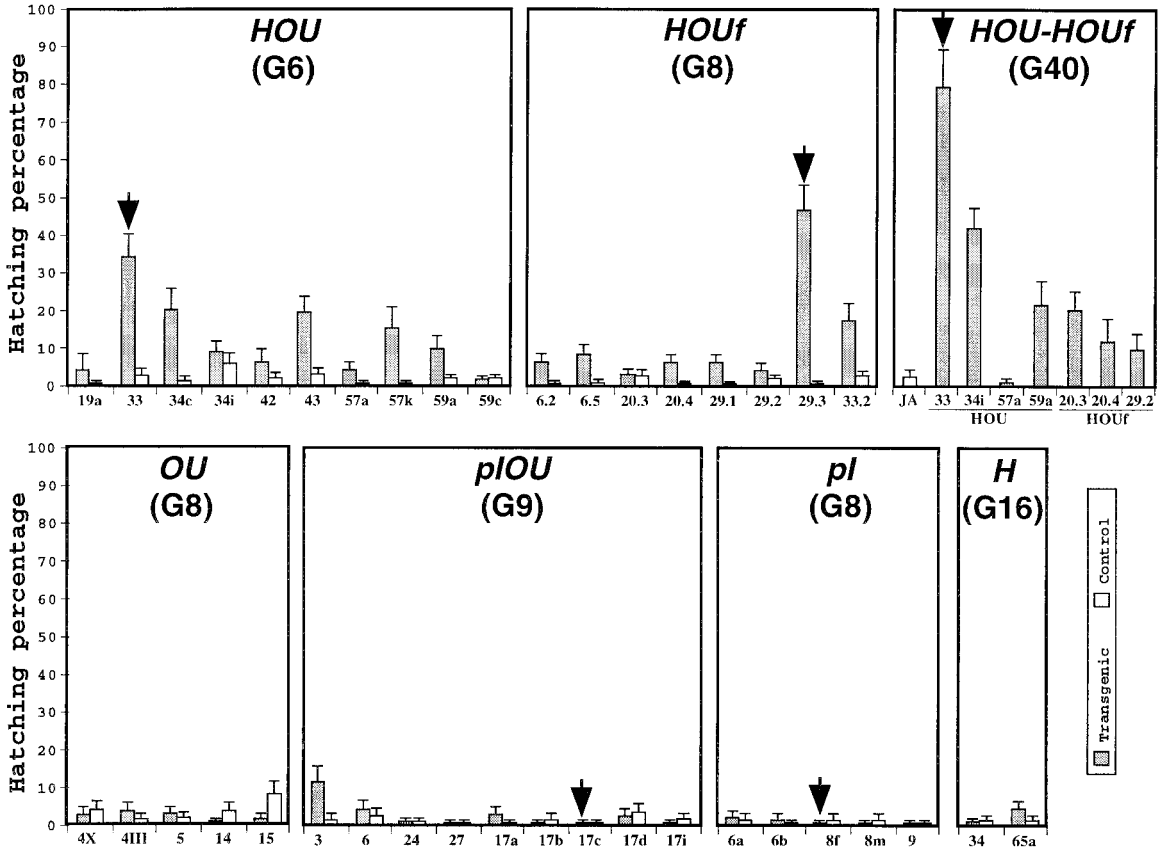

FIGURE 4.—Repressor effect of various transgenes in the *JA* background. Hatching percentages of eggs laid by SF females issued from crosses between males from the w^{1118} inducer stock and females from transgenic lines (shaded bars) or females from sibling nontransgenic lines (open bars), except for HOU and HOUf lines at generation 40, for which the control was the JA stock. The numbers of generations after transgenesis are indicated in each graph. Arrowheads point to transgenic lines containing two homozygous transgenes.

ORF1 under the control of the *I* factor promoter has no apparent regulatory effect: The *hsORF1* transgenes containing ORF1 under the control of a heterologous promoter are artificial and the regulatory effects obtained with these transgenes might be irrelevant to actual *I* factor regulation. We therefore decided to test whether ORF1 under the control of the *I* factor promoter displays a similar repressor activity. Transgenic lines *pIOU* (containing the *pIORF1* transgene) and *pI* (containing the *pI* transgene alone, see Figure 1) were established by *P*-mediated transformation of the *JA* strain. They all contain one homozygous copy of the transgene, except *pIOU17c* and *pI8f*, which contain two. Nontransgenic sibling lines were also established as controls as described above and the regulatory effects were studied at generations 5, 8, 9, 12, and 13 after transgenesis. Typical data are shown in Figure 4. Females of *pIOU* and *pI* transgenic lines produced SF females displaying the same high level of sterility as females of the nontransgenic lines, indicating that ORF1 under the control of the *I* factor promoter has no repressor effect in these conditions. We verified by Northern blot analyses of total RNAs extracted from ovaries of *pIOU* transgenic lines that ORF1 transcripts are actually produced in these flies (data not shown).

The repressor effect of *hsORF1* transgenes is independent from transcription orientation: The transgene *hsORF1as* is similar to *hsORF1*, except that ORF1 is placed in antisense orientation under the control of the *hsp70* promoter and that the *hsp70* 3′ UTR is deleted (see Figure 1). Two lines, named *HOUas49* and *HOUas51*, each containing two homozygous copies of this transgene, were obtained in the *JA* strain. Females of *HOUas* lines produced SF females that were more fertile than SF females produced by females of the *JA* strain. This repressor effect was not affected by heat-shock treatments applied every day during two successive generations according to the protocol described for *HH* lines (Figure 6). Therefore, transgenes that contain ORF1 under the control of the *hsp70* promoter, whatever the orientation of ORF1, display similar regulatory properties on *I* factor activity.

Maternal transmission of the repressor effects of the transgenes: Since *I* factor autoregulation has a maternal component, evidenced by the fact that *I* factor activity is lower in the germ line of RSF females (having received

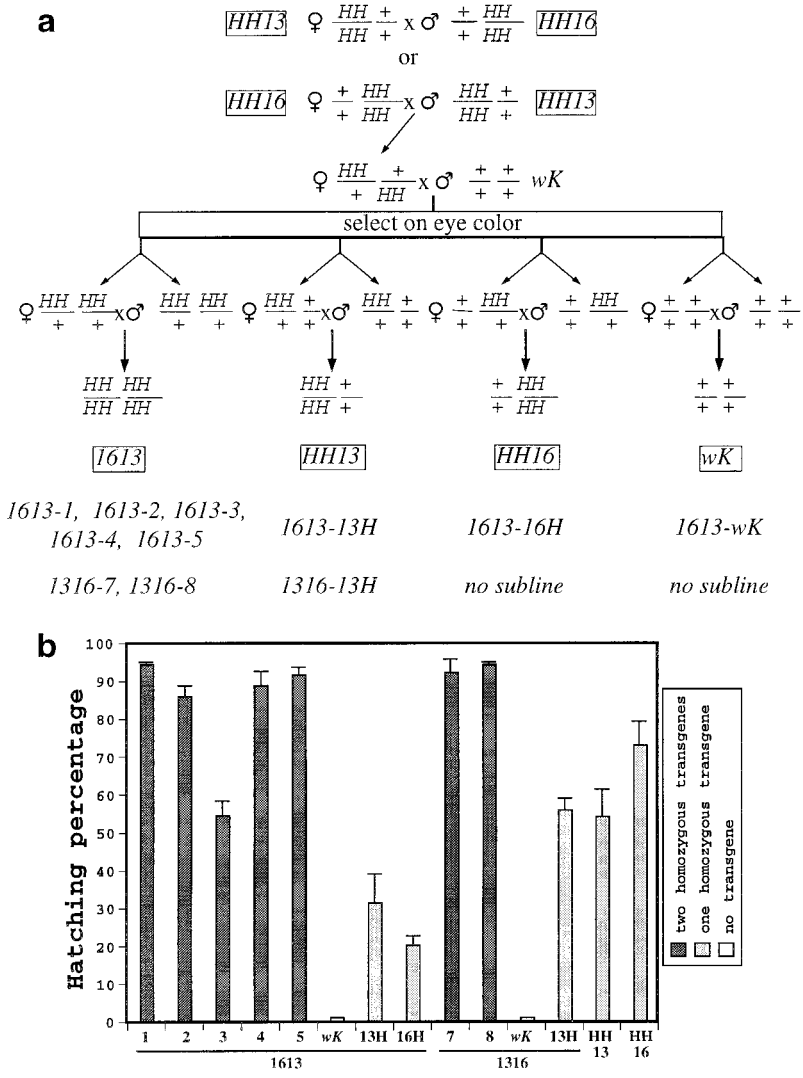

FIGURE 5.—Influence of the copy number of *hsORF1* transgenes. (a) Scheme of crosses used to obtain 1613 and 1316 sublines containing two homozygous transgenes. For clarity only autosomes II and III are indicated. Flies of the various genotypes were recognized by their eye colors since the eyes of flies from the *HH13* and *HH16* lines are of slightly different orange tones, and the double homozygotes *1613* and *1316* have red eyes. (b) Hatching percentages of the eggs laid by SF females issued from crosses between females from the *1613* or *1316* sublines three generations after transgenesis and males from the w^{1118} inducer stock. Standard errors for three samples of sibling SF females are indicated as vertical bars.

I factors from their mother) than in the germ line of SF females (having received *I* factors from their father), we tested whether the regulatory effects of the transgenes are transmitted maternally. For this, we performed the experiments described in Figure 7 with transgenic lines *HOU33*, *HOUf29.3*, and *HOUas51r*. Heterozygous females were produced by the two reciprocal crosses between flies from transgenic lines and flies from the *JA* stock (labeled ♀ and ♂ in Figure 7) and were crossed with males from the w^{1118} inducer stock to produce SF females whose fertility was measured. The fertility of SF females issued from crosses of females from the homozygous transgenic lines (labeled ♂♀ in Figure 7) or females from the *JA* stock (labeled ø in Figure 7) with males from the w^{1118} inducer stock was determined in parallel. In each case a clear maternal effect was observed: heterozygous females having received the transgene from their mothers produced SF females that were more fertile than heterozygous females having received the transgene from their fathers. A zygotic effect is also observed, since the progeny of heterozygous orange-eyed SF females (that received the transgene) were more fertile than that of white-eyed SF females (that did not receive the transgene).

DISCUSSION

In this article we test the ability of various transgenes containing ORF1 sequences to reduce *I* factor activity. Our experimental conditions were designed to detect repressor effects of transgenes on the intensity of the characteristic syndrome of sterility that is associated with high levels of *I* factor transposition. Not surprisingly, this methodology produces highly variable data. The first reason is inherent to any experiment that is based on transgenesis. The activity of a given transgene will obviously be modulated by surrounding regions, depending on its position in the genome. This is why we have systematically studied several transgenic lines for each construct. The second reason comes from the fact that *I* factor activity is modulated by the cellular state, called reactivity, that is prevalent in reactive stocks used

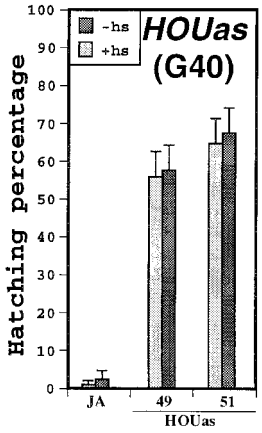

FIGURE 6.—Repressor effect of hsORF1as transgenes. Hatching percentages of the eggs laid by SF females issued from crosses between females from the *HOUas* transgenic lines obtained from the *JA* reactive stock, raised at 23°, and submitted or not every day to a heat treatment during two successive generations, 40 generations after transgenesis, and males from the w^{1118} inducer stock.

to produce SF females. Reactivity is subject to variations due to environmental and physiological factors such as temperature and aging, and these changes are partially heritable for a few generations (BUCHETON and PICARD 1978; BUCHETON 1978, 1979a,b; BUCHETON and BREGLIANO 1982). Even highly controlled laboratory conditions cannot ensure a strict homogeneity of fly culture parameters over many generations. For this reason, we have systematically tested the repressor effect of transgenes at several generations to ensure the reproduceability of the results.

Our data show that various transgenes containing ORF1 sequences under the control of the *hsp70* promoter have a repressor effect on *I* factor activity. This repressor effect is observed with intact ORF1 sequences (transgenes hsORF1 in lines *HH* and *HOU*) as well as when a frameshift mutation prevents synthesis of the ORF1 protein (transgene hsORF1fs in lines *HOUf*) or when ORF1 is in an antisense orientation (transgene hsORF1as in lines *HOUas*). It is abolished when the *hsp70* promoter is removed (transgene ORF1 in lines *OU*). These features mostly coincide with those reported by JENSEN *et al.* (1999a,b). JENSEN *et al.* (1999b) have suggested the involvement of a homology-dependent *trans*-silencing process depending upon the production of an RNA containing ORF1 sequences. Interestingly, recent reports indicate that mobility of *Tc*1 and of other DNA transposons in *Caenorhabditis elegans* can be regulated by a mechanism known as RNA interference (RNAi), which leads to the inhibition of gene function through double-stranded RNA interactions (KETTING *et al.* 1999; TABARA *et al.* 1999). Double-stranded RNA molecules have been shown to be capable of repressing homologous endogenous genes in *D. melanogaster* (KENNERDELL and CARTHEW 1998; MISQUITTA and PATERSON 1999; TUSCHL *et al.* 1999), suggesting that post-transcriptional mechanisms involving RNA intermediates may also occur in this species. It is tempting to speculate the involvment of RNAi in the regulation of transposable elements, including non-LTR retrotransposons in *D. melanogaster*.

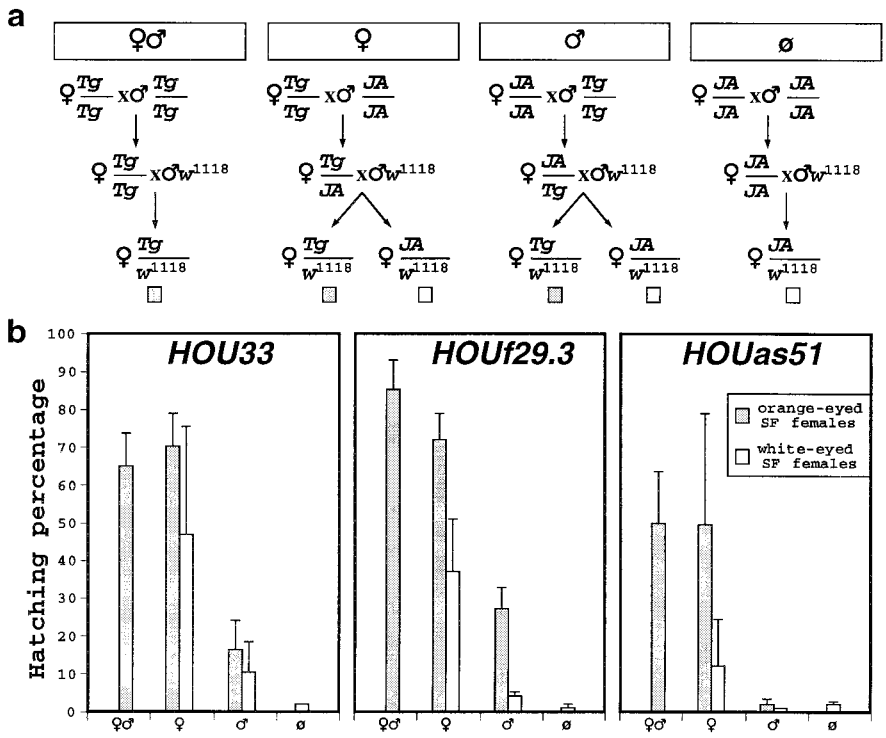

FIGURE 7.—Maternal transmission of repressor effects. (a) Scheme of crosses used to test maternal transmission. Tg stands for transgene and represents *hsORF1* (line *HOU33*), *hsORF1fs* (line *HOUf29.3*), or *hsORF1as* (line *HOUas51*). (b) Hatching percentages of the eggs from SF females issued from crosses between transgenic homozygous (♂♀), transgenic heterozygous (♀ and ♂), or nontransgenic (ø) females and males from the w^{1118} inducer stock. Standard errors for three samples of sibling SF females of the same genotype are indicated as vertical bars.

However, two of our observations are apparently not in agreement with the hypothesis of RNA mediation. First, transgenes containing ORF1 under the control of the *I* factor promoter (transgene pIORF1 in lines *pIOU*) have no regulatory effect, although they are transcribed in the ovaries. Second, while the repressor effect of *hsORF1* transgenes is enhanced by an increase of their copy number (sublines *HH1316* and *HH1613*), we find that whatever the orientation of ORF1, it is not enhanced by heat induction of the *hsp70* promoter, although this promoter is heat inducible in the female germ line at the time of *I* transposition (Busseau *et al.* 1998). This indicates that, although regulation by *hsORF1* constructs is dependent on the copy number of transgenes, it appears not to be correlated to the amounts of their transcripts. Thus, if an RNA intermediate is required for mediating repression by transgenes containing ORF1 sequences, its production is certainly not a sufficient condition. Maybe some specific characteristics of RNAs produced by hsORF1 transgenes could trigger *trans*-silencing. These RNAs differ at their 5′ ends from those produced by pIORF1 transgenes due to distinct transcription initiation from the *hsp70* and the *I* factor promoters (Figure 1) and might have different properties.

It is possible that a DNA-based recognition mechanism could also be invoked: indeed, our results show some similarities to cosuppression involving the *Adh* and *white* genes in *D. melanogaster* (Pal-Bhadra *et al.* 1997, 1999): the presence of multiple *w-Adh* transgenes in the genome can repress the activity of the endogenous *Adh* gene in a copy-number-dependent manner (Pal-Bhadra *et al.* 1997, 1999). This phenomenon is triggered by homology recognition at the DNA level of a nontranscribed segment of the *Adh* regulatory region. It is dependent on proteins of the Polycomb group, implying that changes in chromatin accessibility are involved. It is now important to determine whether the repressor effect of *hsORF1* transgenes on *I* factor activity that we describe here involves proteins of the Polycomb group as well.

Some transgenes show no effect on *I* factor activity in our experimental conditions. However, these conditions would not have allowed us to detect low or slowly accumulating effects. Jensen *et al.* (1999a,b) have shown that the repressor effect of hsORF1 transgenes increases over generations. We observe this phenomenon with *HOU* and *HOUf* transgenic lines, which produce SF females that are less sterile at generations 40–42 than at generations 6–8. We did not study *OU*, *pIOU*, *pI*, and *H* lines after generation 13; therefore, we would not have noticed a low repressor effect slowly accumulating over many generations. Besides, we used transgenic lines that contained only one or two homozygous transgenes, so we would not have detected repressor effects induced by multiple copies of a given transgene. For example, we report here that one or two copies of the 5′ UTR have no detectable regulatory effect (lines *pI*), whereas Chaboissier *et al.* (1998) showed that transgenes containing two or three tandem repeats of the 5′UTR of the *I* factor repress *I* factor activity with efficiencies increasing with their copy number. Taking all this into consideration, we do not exclude the possibility that *pIORF1* or *ORF1* transgenes might have an effect on *I* factor activity that we would not have observed under our experimental conditions, but that could be detected after many generations or in the presence of multiple copies of the transgenes. Even in this case this would indicate that this effect is weaker than that observed with *hsORF1* transgenes. The regulatory effects reported here and by Jensen *et al.* (1999a,b) are associated with the presence of the *hsp70* promoter. This suggests that the presence of the *hsp70* promoter is important although it may not act simply through RNA production. In previous work, we have shown that an element defective in ORF2, *IviP2*, has no repressor effect on *I* factor activity (Busseau *et al.* 1998; I. Busseau, unpublished results). On the contrary, a similar defective element, *IΔZ*, displays a strong repressor effect on *I* factor activity (Jensen *et al.* 1995). *IΔZ* contains a *hsp70-LacZ* reporter gene inserted within ORF2 (Jensen *et al.* 1994), whereas *IviP2* contains the *D. melanogaster vermilion* gene and is devoid of the *hsp70* promoter (Chaboissier *et al.* 1995). It is conceivable that a particular sequence on the *hsp70* promoter is used as a recognized motif for chromatin-binding proteins or is involved in chromatin remodeling.

This raises the question of the relevance of our results on *I* factor self-regulation: indeed, *pIORF1* transgenes are more similar to *I* elements than *hsORF1* transgenes are, since they possess the 5′ UTR of the *I* factor instead of the *hsp70* promoter. There is no evidence that one copy of the *I* factor in the absence of the *hsp70* promoter would be sufficient to trigger repression. Maternal transmission is one characteristic of natural repression of *I* elements occurring in inducer strains. Our study shows that the repressor effect of *hsORF1* transgenes on *I* factor activity is maternally transmitted, suggesting that our observations are relevant to *I* self-regulation. The regulation of the *I* factor is complex and still not understood. It might actually involve more than one mechanism. Whatever the case it is a very stimulating question that can be used to study epigenetic inheritance.

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