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# Functional dissection of the *Drosophila* modifier of variegation *Su(var)3-7*

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## SUMMARY

**An increase in the dose of the heterochromatin-associated Su(var)3-7 protein of *Drosophila* augments the genomic silencing of position-effect variegation. We have expressed a number of fragments of the protein in flies to assign functions to the different domains. Specific binding to pericentric heterochromatin depends on the C-terminal half of the protein. The N terminus, containing six of the seven widely spaced zinc fingers, is required for binding to bands on euchromatic arms, with no preference for pericentric heterochromatin. In contrast to the enhancing properties of the full-length protein, the N terminus half has no effect on heterochromatin-dependent position-effect variegation. In contrast, the C terminus moiety suppresses variegation. This dominant negative effect on variegation could result from association of the fragment with the wild type endogenous protein. Indeed, we have found and mapped a domain of self-association in this C-terminal half. Furthermore, a small fragment of the C-terminal region actually depletes pericentric heterochromatin from endogenous Su(var)3-7 and has a very strong suppressor effect. This depletion is not followed by a depletion of HP1, a companion of Su(var)3-7. This indicates that Su(var)3-7 does not recruit HP1 to heterochromatin. We propose in conclusion that the association of Su(var)3-7 to heterochromatin depends on protein-protein interaction mediated by the C-terminal half of the sequence, while the silencing function requires also the N-terminal half containing the zinc fingers.**

Key words: *Drosophila melanogaster*, Position-effect variegation, Heterochromatin

## INTRODUCTION

In *Drosophila melanogaster*, position-effect variegation (PEV) occurs when the relocation or insertion of a gene next to blocks of pericentric heterochromatin results in a mosaic phenotype (Spofford, 1976; Weiler and Wakimoto, 1995). The extent of the silencing depends both on *cis*-DNA sequences, and on *trans*-acting proteins encoded by suppressors or enhancers of PEV. Some loci exhibit both a haplo-suppressor and a triplo-enhancer effect on PEV, making them good candidates for being structural components of constitutive heterochromatin (Reuter and Spierer, 1992; Wallrath, 1998). Among them, the *Su(var)3-7* gene encodes a protein associated mostly with pericentric heterochromatin in embryonic nuclei and in salivary gland polytene chromosomes; it also binds telomeres and a few euchromatic sites (Delattre et al., 2000). The large protein of 1169 amino acids contains seven atypical and widely spaced zinc fingers motifs in its N-terminal moiety (Cléard et al., 1995; Reuter et al., 1990). Su(var)3-7 domains containing zinc fingers bind DNA in vitro (Cléard and Spierer, 2001). The C-terminal moiety contains a motif of 40 amino acids (amino acids 906-945) identified in the conserved domain database as the BESS motif (Altschul et al., 1997). This motif is named after the proteins in which it is found, namely BEAF (Zhao et al., 1995), Su(var)3-7 and Stonewall (Clark and McKearin, 1996). To date, this motif is known in 19 proteins, all from *Drosophila melanogaster*.

Another component of constitutive heterochromatin in *Drosophila* is the protein HP1 encoded by the *Su(var)2-5* gene (Eissenberg et al., 1990; Eissenberg et al., 1992). On polytene chromosomes, and as Su(var)3-7, HP1 is associated with pericentric heterochromatin, telomeres and some euchromatic sites (Fanti et al., 1998; James et al., 1989; Kellum and Alberts, 1995). HP1 contains two main domains, the chromodomain and the chromoshadow domain. These domains are conserved in the HP1 orthologues reported in mammals (Saunders et al., 1993; Singh et al., 1991; Wreggett et al., 1994) and in the two paralogues recently found in *Drosophila melanogaster* (Smothers and Henikoff, 2001). The chromoshadow domain of mouse HP1 orthologues is required for protein-protein interactions and dimerisation (Brasher et al., 2000; Jones et al., 2000; Nielsen et al., 2001). Dimerisation has also been seen for the HP1 homologue Swi6 of yeast (Cowieson et al., 2000). In mammals, HP1 is found associated with Suvar39H1, the orthologue of the *Drosophila* modifier of PEV Su(var)3-9 (Tschiersch et al., 1994; Aagaard et al., 1999). Suvar39H1 contains a SET domain adjacent to a cystein-rich region carrying a histone methyl transferase activity (Rea et al., 2000). Suvar39H1 specifically methylates the lysine 9 of histone H3, and HP1 recognises and associates with this methylated histone (Bannister et al., 2001; Lachner et al., 2001; Jacobs et al., 2001). *Drosophila* HP1 also interacts with Su(var)3-7, as shown by the two hybrid assay in yeast (Delattre et al., 2000), by co-immunoprecipitation from nuclear extract, and by

recruitment of Su(var)3-7 by delocalised HP1 (Cléard et al., 1997; Delattre et al., 2000). These data suggest that HP1, Su(var)3-9 and Su(var)3-7 are associated in heterochromatin.

To investigate in vivo the function of the different Su(var)3-7 domains, we have expressed tagged fragments of Su(var)3-7 in the fly. We have analysed the chromosomal localisation of these fragments, their effect on position-effect variegation, and their ability to modify endogenous HP1 localisation. The data obtained lead us to propose a model for the function of Su(var)3-7 in the genomic silencing of position-effect variegation.

## MATERIALS AND METHODS

### HA:Su(var)3-7 constructs

Fragments of the *Su(var)3-7* cDNA (Cléard et al., 1995) were cloned in phase downstream of the haemagglutinin tag (HA) using the pHA vector of N. Hulo and V. Pirrotta (unpublished). HA:Su(var)3-7 fragments were inserted in a modified version of the C4-*yellow* transformation vector (Sigrist and Pirrotta, 1997). Constructs were made of the following fragments: a 3.4 kb fragment corresponding to amino acids 37-1169 for the construct HA:FL; a 2.15 kb fragment (aa 37-753) for HA:1-6; a 2.4 kb fragment (aa 363-1169) for HA:4-Ct; a 2.1 kb fragment (aa 485-1169) for HA:5-Ct; a 1.7 kb fragment (aa 614-1169) for HA:6-Ct; a 1.3 kb fragment (aa 737-1169) for HA:7-Ct; a 1.2 kb fragment (aa 755-1169) for HA:Δ7-Ct; a 0.6 kb fragment (aa 755-970) for HA:Δ7-B and a 0.6 kb fragment (aa 971-1169) for the HA:B-Ct construct.

### Western blot analysis

Ten males and ten females from each transgenic line were heat-shocked for 30 minutes. After 1 hour of recovery, flies were homogenised in 200 µl of protein homogenisation buffer (8 M urea, 100 mM $NaH_2PO_4$, 10 mM Tris-HCl pH 6.4). 200 µl of sample buffer (4% SDS, 17.5% glycerol 120 mM Tri-HCl pH 6.8 and 0.01% bromophenol blue) were added and the samples were boiled for 5 minutes before western analysis. Proteins were stained with an anti-HA monoclonal antibody diluted 1:100, and detected with an anti-mouse IgG-alkaline phosphatase-conjugated antibody diluted 1:2000.

### Two-hybrid interaction trap in yeast

Screens and tests were performed as described by Delattre et al. (Delattre et al., 2000).

### Immunostaining of polytene chromosomes

Third instar larvae were heat shocked 15 minutes at 37°C, unless specified otherwise, and allowed to recover at room temperature for 1 hour before squashing. Procedures for immunostaining were as described previously (Platero et al., 1995). Anti-HA and anti-Su(var)3-7 antibodies were used at a dilution of 1:100, and anti-HP1 (CI49, a gift from Sarah Elgin) at 1:400.

### Effect of HA:Su(var)3-7 mutant proteins on variegation

To test the effect of the heat-shock induction of HA:Su(var)3-7 proteins on the $w^{m4h}$ and *Heidi* lines (Seum et al., 2000), females bearing the variegated alleles were crossed with males homozygous for the *HA-Su(var)3-7* transgene, and with *yw* males as control. Heat shock was carried out by incubating embryos at 30°C until the beginning of the third instar larval stage, and then by administrating three 30-minute heat shocks at 37°C per day until adult emergence. Eye pigment measurements of males were made according to the method of Sun and coworkers (Sun et al., 2000).

## RESULTS

### Tagged fragments of Su(var)3-7 are expressed in flies

To determine the function of the domains of Su(var)3-7, we have made transgenic flies expressing different fragments of the protein. The Su(var)3-7 fragments were tagged by an haemagglutinin epitope (HA), and their expression placed under control of a heat shock promoter. Fig. 1 shows the alignment of the different constructs with the wild-type Su(var)3-7 protein sequence as well as the motifs previously reported in Su(var)3-7. The figure also shows the blots of crude protein extracts from adult flies expressing the different tagged proteins. The blots stained with an anti-HA antibody show that the proteins are easily detected at the right size in extracts from heat-shocked flies, but are undetectable in non-heat-shocked flie extracts. This demonstrates that all fragments can be conditionally expressed, and are readily detected by their tag.

### The C-terminal part of Su(var)3-7 is required for heterochromatin-specific association

On polytene chromosomes, expression of the full-length tagged Su(var)3-7 (HA:FL) caused by to the leakiness of the hsp70 promoter in the absence of heat shock, gives strong staining of pericentric heterochromatin (Fig. 2A). Over-

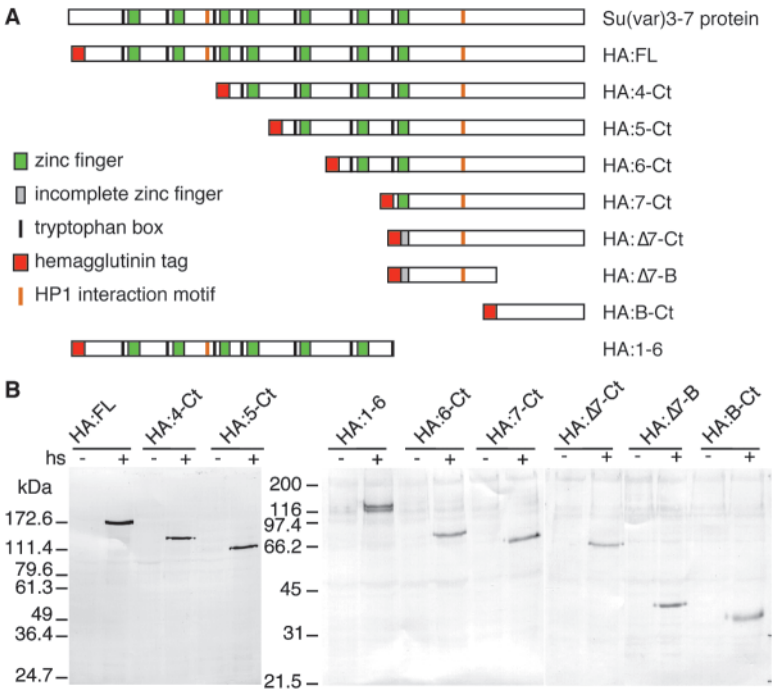


**Fig. 1.** Tagged Su(var)3-7 constructs. (A) Alignment of the tagged Su(var)3-7 constructs. (B) Western blot of crude protein extracts from heat shocked and non-heat shocked adult flies expressing different tagged constructs. Detection with an anti-HA antibody. (Left) 8% polyacrylamide gel; (right) 10% polyacrylamide gel.

expression of HA:FL resulting from 15 minutes heat shock at 37°C results in additional staining of the euchromatin arms with a banding pattern different from DAPI staining of DNA (Fig. 2B). The same pattern was seen for over-expressed *Su(var)3-7* cDNA (Delattre et al., 2000). This means that tagging the protein at its N terminus with the HA epitope does not affect its chromatin binding properties. In contrast, the HA:1-6 construct, containing the N-terminal half of Su(var)3-7 with six of the seven zinc fingers, does not specifically concentrate at pericentric heterochromatin (Fig. 2A). The chromosomes are stained in a pattern that coincides with the DAPI staining (Fig. 2B), and the protein does not show preference for pericentric heterochromatin. This suggests that the heterochromatic specificity is not given by the zinc fingers moiety of Su(var)3-7, but requires the C-terminal region. To verify this assumption, we have analysed chromosomes of larvae expressing the tagged C-terminal tail of Su(var)3-7 (HA:Δ7-Ct). This construct does not comprise zinc fingers. Staining is specifically and exclusively seen at pericentric heterochromatin (Fig. 2A). This set of experiments suggest that the C-terminal moiety of Su(var)3-7, though devoid of zinc fingers, is responsible for the targeting of the protein to heterochromatin.

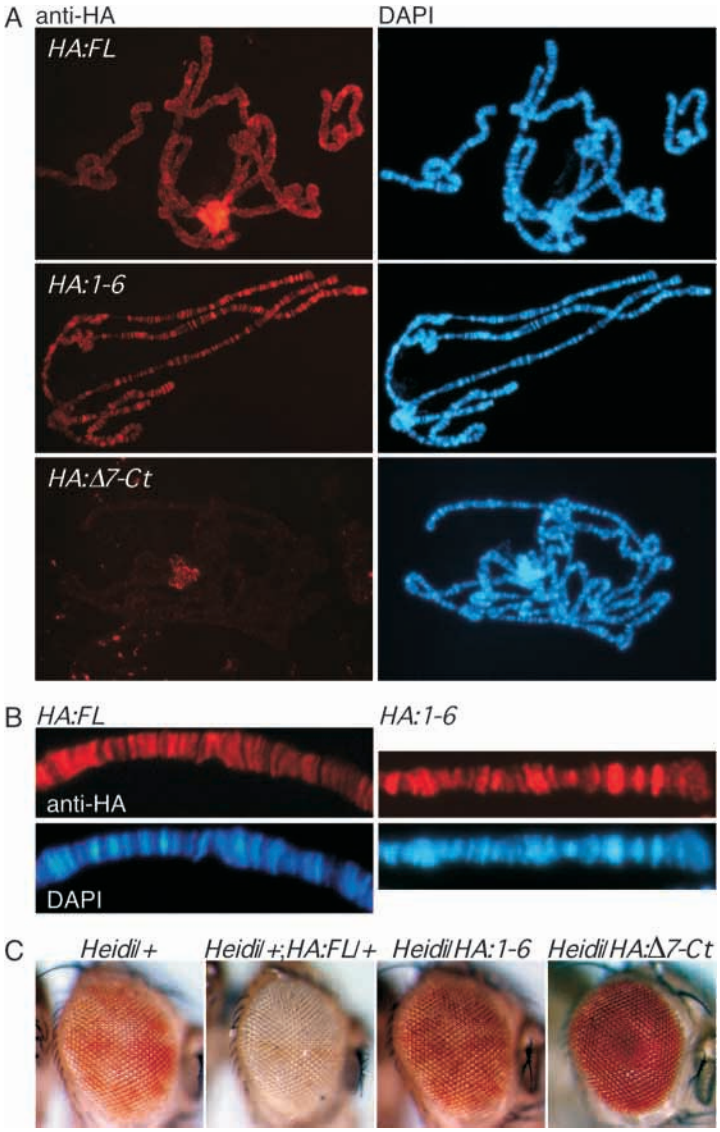


**Fig. 2.** Chromosomal localisation and effect on PEV of the N- and C-terminal parts of Su(var)3-7. (A) Polytene chromosomes from salivary glands of transgenic larvae expressing the HA:FL, HA:1-6 or HA:Δ7-Ct constructs. Staining with an anti-HA antibody detected with a Cy3-labelled secondary antibody (red) and DAPI (blue). HA:FL and HA:1-6 did not require heat-shock induction for detectable expression. (B) Banding pattern of polytene chromosomes. HA:FL was heat shocked to detect a banding pattern. This was not necessary for HA:1-6. Staining is as in A. (C) Effect on *Heidi* variegation of the different tagged constructs. Eyes of heat shocked males from crosses between *w*; *Heidi/CyO* females and *yw/Y; P{y+, HA:Su(var)3-7}* males. Corresponding eye pigment measurements, normalised to the control *Heidi/+*:*Heidi/+* = 100±5.4; *Heidi*/+; HA:FL/+ = 49±4.5; *Heidi/HA:1-6* = 94±6.4; *Heidi/HA:Δ7-Ct* = 240±13.

## The C-terminal part of Su(var)3-7 suppresses variegation and interacts with itself

We have examined the effect of the expression of the two tagged halves of Su(var)3-7 on PEV. Transgenic flies expressing these constructs were crossed with both the *Heidi* and the classical $w^{m4h}$ variegating lines. *Heidi* contains a $P\{w^+\}$ euchromatic transgene relocated in heterochromatin as a result of a X-ray induced chromosomal inversion (Seum et al., 2000). $w^{m4h}$ is itself the result of an inversion in the X chromosome relocating the *white* gene ($w^+$) next to pericentric heterochromatin. First, and as hoped for, we found that heat-shock induced expression of the full-length protein (HA:FL) enhances heterochromatin mediated repression of the *white* gene in *Heidi* flies (Fig. 2C). The same result was obtained with $w^{m4h}$ (not shown). This confirms the enhancer effect of extra doses of the *Su(var)3-7* gene (Reuter et al., 1990). In contrast, the N-terminal construct (HA:1-6) containing six of the seven zinc fingers had no effect on PEV as shown in Fig. 2C. This is consistent with the loss of its specific association with pericentric heterochromatin on polytene chromosomes as reported above (Fig. 2A). In contrast to the enhancer effect of the full-length protein, the tagged C-terminal moiety (HA:Δ7-Ct), which binds specifically to pericentric heterochromatin, had a suppressor effect on PEV (Fig. 2C).

One explanation for the suppressor effect on PEV of the C-terminal half of Su(var)3-7 could be its ability to form an inactive complex with endogenous Su(var)3-7 or a protein interacting with it. We have used the yeast two-hybrid protein interaction trap system (Fields and Song, 1989) to search for proteins interacting with Su(var)3-7. A segment of Su(var)3-7 extending from the seventh zinc finger to the C-end of the protein, namely amino acids 736-1169, was used as a bait to screen a cDNA library derived from *Drosophila* embryos (Materials and Methods). About three million yeast transformants were screened, and 50 positive clones isolated. One of them, named α21, encodes a fragment of Su(var)3-7 itself, corresponding to amino acids 808 to 991. To delimit the domain of Su(var)3-7 involved in its self-association, we performed a deletion analysis illustrated in Fig. 3. The zinc finger-containing region was found not to be involved in this interaction. Only fragments containing the C-terminal part of Su(var)3-7 interact with α21. The smallest

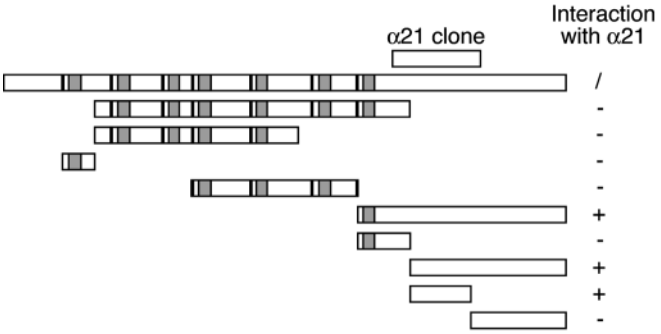


**Fig. 3**. Su(var)3-7 interacts with itself in the yeast two-hybrid assay. α21 is a fragment of Su(var)3-7 that uses Su(var)3-7 itself as bait in a two-hybrid screening of a *Drosophila* embryonic cDNA library. A schematic representation of α21 is given above the wild-type protein. A series of Su(var)3-7 deletion constructs have been tested to precisely map the domain of interaction of Su(var)3-7 with itself. /, impossible to test (Delattre et al., 2000); +, interaction with α21; –, no interaction. Grey boxes: zinc fingers; black bars: tryptophan boxes.

construct tested that was capable of promoting self-association of Su(var)3-7 contained amino acids 845 to 971. This construct also interacts with HP1 (Delattre et al., 2000), but fails to interact with unrelated proteins, or with the empty pJG4-5 vector (data not shown). As the fragment promoting self-association contains the BESS motif (amino-acids 906-945), we surmise that this motive is responsible for Su(var)3-7 dimerisation.

If Su(var)3-7 associates with itself in vivo, we can then ask whether the construct comprising the interaction region (Δ7-Ct) is able to delocalise the endogenous protein. Fig. 2A, and Fig. 4A show that the construct expressing the C-terminal third of Su(var)3-7 (HA:Δ7-Ct) stains the chromocenter of polytene chromosomes in the form of spots. The localisation of endogenous Su(var)3-7 is indeed modified in this context. It also takes the form of spots, and these spots co-localise with the tagged fragment HA:Δ7-Ct (Fig. 4A).

## A C-terminal fragment depletes wild type Su(var)3-7 from polytene chromosome

To delineate more precisely the domain in the C-terminal part of Su(var)3-7 capable of delocalising the endogenous protein, we expressed two fragments of the C-terminal part of Su(var)3-7. These fragments, HA:Δ7-B and HA:B-Ct, are schematised in Fig. 1. Interestingly, they do not bind polytene chromosomes (data not shown) although they are readily detectable on western blot (Fig. 1) and in the nucleoplasm of whole-mount salivary glands cells (not shown). As the C-terminal moiety of Su(var)3-7 associates with itself, it was also of interest to see whether the two fragments of the C-terminal end modify the localisation of endogenous Su(var)3-7. Polytene chromosomes from larvae expressing HA:Δ7-B or HA:B-Ct constructs were stained simultaneously with anti-Su(var)3-7 and anti-HA. The localisation of endogenous Su(var)3-7 is not affected in chromosomes of HA:B-Ct expressing larvae (not shown), but Su(var)3-7 staining disappears from polytene chromosomes of HA:Δ7-B (see below as part of other experiments in Fig. 7). We interpret this result as meaning that the Δ7-B fragment recruits endogenous Su(var)3-7 away from chromosomes.

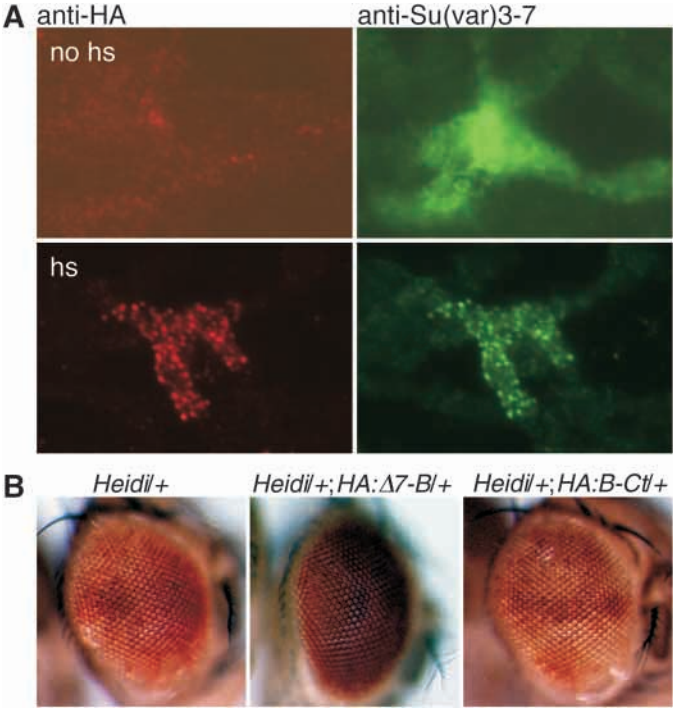


**Fig. 4.** The C-terminal part of Su(var)3-7 recruits the endogenous protein and suppresses PEV. (A) Double staining of polytene chromosomes from larvae expressing the HA:Δ7-Ct protein with an anti-HA antibody (red) and anti-Su(var)3-7 antibody (green). The anti-Su(var)3-7 antibody was raised against a N-terminal fragment of the protein, and permits the detection of the endogenous protein but not of the HA:Δ7-Ct protein. hs, heat shocked (B) Effect of different tagged constructs on *Heidi* variegation. Corresponding eye pigment measurements normalised to the control *Heidi/+: Heidi/+* = 100±5.3; *Heidi/+; HA:Δ7-B/+* = 881±103; *Heidi/+; HA:B-Ct/+* = 88±4.4.

We next tested the possible effect on variegation of the depletion of Su(var)3-7 from pericentric heterochromatin. HA:Δ7-B does indeed a strong suppressor effect on PEV while HA:B-Ct has no effect (Fig. 4B). We conclude that the Δ7-B region contains a domain recruiting endogenous Su(var)3-7 and depleting it from the chromocenter, with the consequence of suppressing PEV.

We then tested whether, reciprocally, the over-expression of wild-type Su(var)3-7 delocalises the C terminus fragment, namely Δ7-Ct. To do so, we have combined it, under heat shock, with the construct over-expressing the wild-type Su(var)3-7 (Cléard et al., 1995). After heat shock, the over-expressed Su(var)3-7 protein covers the entire chromosome (Fig. 5) (Delattre et al., 2000). In these conditions, the HA:Δ7-Ct protein was found predominantly at the chromocenter, but it also stained weakly, but significantly, the whole of the chromosome arms (Fig. 5). In the wild-type background, its localisation was restricted to the chromocenter (Fig. 5). To allow for comparison of intensity of staining in Fig. 5, glands from both genotypes were placed on the same slide. Therefore, the two nuclei shown went through the same procedures. Finally, the pericentric spots of HA:Δ7-Ct seen in a wild-type background are replaced by a strong, smooth staining when Su(var)3-7 is over-expressed. We conclude that endogenous

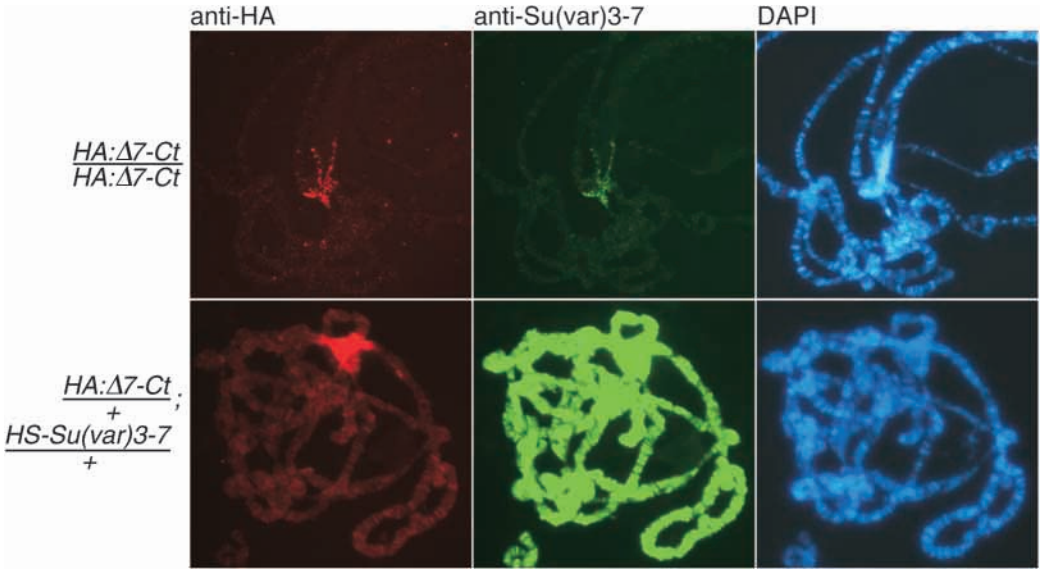


**Fig. 5.** The Su(var)3-7 wild type protein recruits the HA:Δ7-Ct construct. Double staining of polytene chromosomes using an anti-HA antibody (red), an anti-Su(var)3-7 antibody (green), and DAPI (blue). Top: chromosomes of homozygous *yw; HA:Δ7-Ct* larvae; bottom: chromosomes from heterozygous *yw; HA:Δ7-Ct/+; P{HS-Su(var)3-7}/+* larvae. Control and test chromosomes were squashed on the same slide in this experiment to allow for semi-quantitative evaluation.

Su(var)3-7 seems able to recruit the C-terminal construct HA:Δ7-Ct and vice versa.

### The N-terminal part of Su(var)3-7 is required for binding to euchromatin

To analyse the role of the Su(var)3-7 zinc fingers located in the N-terminal half of the protein, we made fly lines expressing Su(var)3-7 fragments containing the C-terminal part plus one to four zinc fingers (HA:7-Ct, HA:6-Ct, HA:5-Ct, HA:4-Ct; summarized in Fig. 6, and data not shown). These constructs are expressed after heat-shock, as seen on western blot (Fig. 1). All the fragments bind pericentric heterochromatin, but the ability to bind euchromatic sites depends on the presence of zinc fingers. In fact, the HA:Δ7-Ct construct, which is devoid of zinc fingers, is unable to bind euchromatic sites, whereas HA:7-Ct, which contains only the seventh zinc finger, stains a number of bands on euchromatic arms, namely those already described for endogenous Su(var)3-7 (Delattre et al., 2000) (data not shown). This result in vivo contrasts with work in vitro showing that the minimum requirement for binding DNA is two zinc fingers (Cléard and Spierer, 2001). The number of zinc fingers does nonetheless play a role. While with two and three zinc fingers, HA:6-Ct and HA:5-Ct give only a discrete number of bands (Fig. 6), HA:4-Ct with its four zinc fingers expands on euchromatic arms with a pattern similar to the over-expressed full-length protein. It is important to note here that protein fragments with one to four zinc fingers suppress PEV, while the only protein having an enhancer effect is the full-length protein (HA:FL).

### HP1 does not recruit Su(var)3-7 fragments missing its N terminus

Platero and co-workers (Platero, 1995) showed that an HP1-Polycomb chimeric protein, containing the chromodomain of Polycomb instead of its own, delocalises endogenous HP1 to Polycomb binding sites. Moreover, the delocalised HP1 recruits endogenous Su(var)3-7 (Delattre et al., 2000). To delineate more precisely the domain of Su(var)3-7 causing this delocalisation, we have expressed together in flies Su(var)3-7 fragments and the HP1-Polycomb chimera. None of the fragments tested is recruited at Polycomb binding sites (not shown). In a previous study, we mapped, by the two hybrid assay, three domains of Su(var)3-7 involved in the interaction with HP1 (Delattre et al., 2000): the first one between the second and the third zinc finger, the second one between the fifth and the sixth and the last one in the C-terminal tail of Su(var)3-7. That constructs containing one or two regions of interaction are not delocalised by the HP1/Pc chimera, suggests that the Su(var)3-7-HP1 interaction depends on the presence of more than one or two domains of interaction with HP1. Unfortunately, owing to their spreading properties after heat-shock induction, it was not possible to test the delocalisation of mutant proteins containing more than two interaction regions.

Finally, we examined the localisation of endogenous HP1 on polytene chromosomes from larvae expressing fragments of

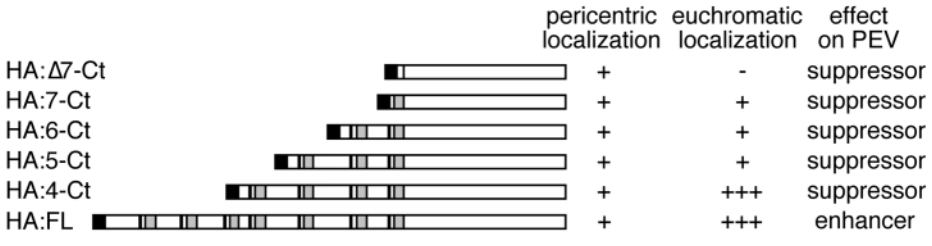


**Fig. 6.** Localisation and effect on PEV of tagged constructs containing different sets of zinc fingers. Localisation was determined by immunostaining of polytene chromosomes. –, no binding on euchromatic arms: +, discrete number of bands on euchromatic arms; +++, band-interband pattern on euchromatic arms. Effect of constructs on PEV were determined by crosses with the *Heidi* variegating line. Black box, HA tag; grey boxes, zinc fingers; light grey boxes, incomplete zinc finger; black bars, tryptophan boxes.

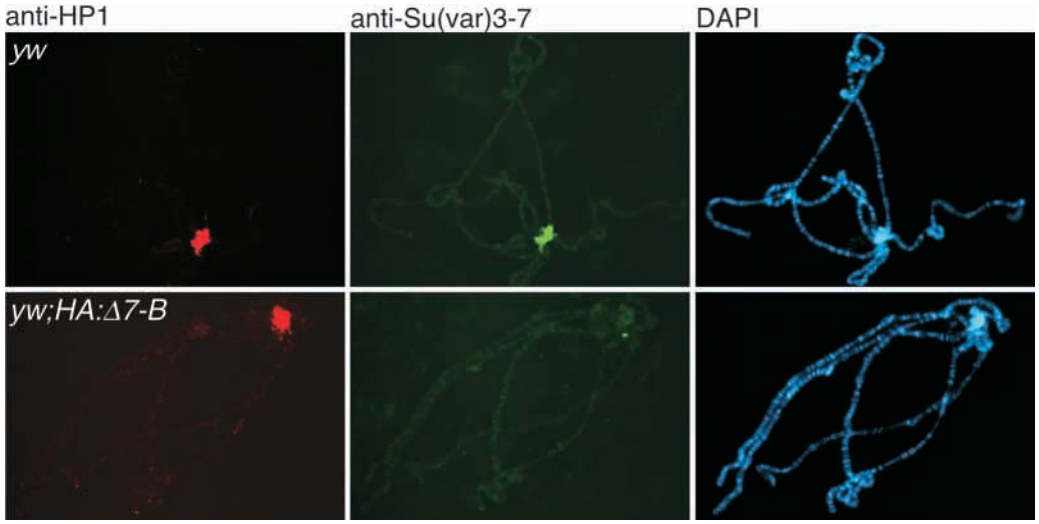


**Fig. 7.** Delocalisation of endogenous Su(var)3-7 by tagged constructs does not modify endogenous HP1 localisation. Staining of polytene chromosomes with an anti-HP1 antibody (red), an anti-Su(var)3-7 antibody (green) and DAPI (blue). (Top) Chromosomes from control *yw* larvae; (bottom) chromosomes from *yw;HA:Δ7-B* larvae.

Su(var)3-7. None of the nine Su(var)3-7-tagged fragments we made was able to recruit HP1 away from its normal pattern, as illustrated for one case in Fig. 7. This figure shows a total depletion of endogenous Su(var)3-7 from pericentric heterochromatin when HA:Δ7-B is expressed. Even in these conditions, HP1 localisation is not modified: staining of pericentric heterochromatin, telomeres and euchromatic bands decorated by HP1 is still visible. Depletion of Su(var)3-7 from the chromocenter does not disturb HP1 localisation.

## DISCUSSION

### Su(var)3-7 associates with itself in vivo

We have shown that Su(var)3-7 interacts with itself in yeast. The interaction domain maps to the C-terminal region (amino acids 845-971, of 1169). We also provide evidence that this interaction takes place in the fly: The HA:Δ7-Ct tagged construct (amino-acids 754 to 1169, of 1169) forms spots in heterochromatin, presumably due to aggregates, and recruits endogenous Su(var)3-7 to these spots. Moreover, the HA:Δ7-B construct (amino-acids 754 to 970), which does not bind polytene chromosomes, depletes endogenous Su(var)3-7 protein from the chromocenter. Both constructs contain the domain of interaction mapped in yeast and are strong suppressors of PEV. The cytology and the phenotype can be explained by a titration of endogenous Su(var)3-7 by the self-association domain of the tagged fragment. Our interpretation is that the sequestration of the endogenous protein by non functional fragments prevents normal interaction of Su(var)3-7 protein with itself, protein or DNA partners, and leads to the decrease of the repression mediated by heterochromatin.

The region of Su(var)3-7 promoting self-association contains the motif BESS (Altschul et al., 1997). Interestingly, among members of the family, the BESS motif is also found in the part of the BEAF protein implicated in the oligomerisation of the protein (Hart et al., 1997). Moreover, and although Su(var)3-7 seems to be a fast evolving protein, the BESS motif is one of the best conserved region in the Su(var)3-7 proteins of *Drosophila melanogaster* and *Drosophila virilis* (our unpublished work). This leads us to propose that the BESS motif is an important domain of Su(var)3-7, ensuring self-association.

The functional significance of this self-association is not known, but it provides a means of forming multimeric complexes promoting heterochromatin formation and associated silencing. Indeed, mammalian HP1 proteins homo- and heteromerise in vivo and in vitro (Le Douarin et al., 1996; Ye et al., 1997; Nielsen et al., 2001). Furthermore, recent in vitro studies have shown that HP1 is required as a dimer for interaction with CAF1 and TIFβ in mice (Brasher et al., 2000).

### The C-terminal part of Su(var)3-7 is required for specific binding to heterochromatin

When over-expressed, not only does Su(var)3-7 bind strongly to heterochromatin, but staining expands through euchromatin. We have shown here that the full-length tagged version of Su(var)3-7 (HA:FL) behaves in an analogous manner. The banding pattern is neither identical nor complementary to DNA staining. HA:FL does not specifically bind all bands or interbands, but it has a yet unknown specificity for a particular DNA or chromatin landscape. In contrast, HA:1-6, the construct containing the first six zinc fingers and lacking the C-terminal half of Su(var)3-7, covers complete polytene chromosomes, without preference for heterochromatin, with a pattern similar to DNA staining. In vitro, fragments containing two or more Su(var)3-7 zinc fingers motifs have a general affinity for DNA, with a preference for some satellite DNA sequences (Cléard and Spierer, 2001). However, the apparent absence of specificity of HA:1-6 association with polytene chromosomes leads us to conclude that in vivo the heterochromatin specificity of Su(var)3-7 is not given by its zinc fingers. Possible preference for satellite DNA in vivo is difficult to assess because of vast underreplication of these sequences in polytene chromosomes.

HA:Δ7-Ct, the construct containing the C-terminal half, specifically binds to pericentric heterochromatin. We cannot exclude that this specific localisation to heterochromatin depends at least in part on protein interaction with endogenous Su(var)3-7. Nonetheless, the in vivo analysis clearly shows that the domain of Su(var)3-7 self-association is not sufficient for

the targeting to heterochromatin. HA:Δ7-B, a short C-terminal segment, contains the dimerisation domain mapped in yeast, does not bind heterochromatin, and depletes pericentric heterochromatin from endogenous Su(var)3-7.

The specificity for pericentric heterochromatin located in the C-terminal half is probably mediated by interaction with another heterochromatic partner. The domain of Su(var)3-7 interacting with this partner should lie around the *Bst*EII restriction site separating the constructs HA:Δ7-B and HA:B-Ct (Fig. 1). These constructs do not bind to chromatin, whereas HA:Δ7-Ct, from which they originate by cleavage with *Bst*EII, does bind.

### Interactions between Su(var)3-7 and HP1

We believe that HP1, although known to bind to Su(var)3-7, is not the partner necessary for the targeting of Su(var)3-7 to heterochromatin through the *Bst*EII site region of the C-terminal part. First, none of the three domains of HP1 interaction mapped in yeast corresponds to this *Bst*EII region. Second, the localisation of the HA:Δ7-Ct construct of Su(var)3-7 as spots in the chromocenter has no effect on HP1 localisation. Reciprocally, ectopic HP1, as a HP1/Pc chimera, does not recruit HA:Δ7-Ct, the C-terminal half of Su(var)3-7, while it does recruit the full-length protein. We infer that the targeting of HA:Δ7-Ct to the chromocenter is due to a yet unidentified partner. And although HP1 does recruit the full-length Su(var)3-7 (Delattre et al., 2000), the constructs containing one or two HP1-binding domains are not delocalised by the HP1/Pc chimera. We therefore propose that the Su(var)3-7-HP1 interaction requires the presence of the three interaction domains together. Finally, when endogenous Su(var)3-7 is totally depleted from the chromocenter by the presence of the HA:Δ7-B construct, HP1 localisation is not at all modified. The evidence above indicates that Su(var)3-7 does not recruit HP1 to heterochromatin.

### A model for the role of Su(var)3-7 in heterochromatin

Over-expression of the full-length Su(var)3-7 protein dramatically enhances variegation. In contrast, over-expression of HA:1-6, the construct with six zinc fingers, has no effect on PEV. This suggests that to be functional in heterochromatin, the zinc fingers domains of Su(var)3-7 need the dimerisation of the protein and/or interaction with partners. Besides, the constructs containing one to four zinc fingers plus the complete C-terminal part do not enhance variegation as the full-length protein does. This means that the number of zinc fingers is also crucial for the function of the protein. The zinc fingers of Su(var)3-7 are not classical C2H2 type zinc fingers because of their non-canonical sequence, an upstream tryptophan motif and of the rather long inter-digital space (Cléard et al., 1995). We propose that the dispersion of Su(var)3-7 zinc fingers in the N-terminal half of the protein allows them to make contact with DNA at a distance. It provides the flexibility required to pack the dispersed bound DNA sequences in a more compact conformation, thus contributing to the condensed heterochromatic conformation and to the repressed state. An analogous function in altering the DNA conformation has been described for the 12 zinc finger proteins encoded by the *Suppressor of Hairy-wing* gene. These zinc fingers directly contact DNA and increase its flexibility together with the C-terminal of the protein, itself not required for DNA binding (Shen et al., 1994).

The Su(var)3-7 protein consisits of two complementary domains: the N-terminal zinc fingers moiety and the C-terminal part. The zinc fingers contribute to the repression function as a DNA compacting tool, while the C-terminal domain promotes dimerisation through the BESS motif and interaction with partners to ensure heterochromatin recognition and association. In position-effect variegation, expansion of the repressive complexes, or sequestration of heterochromatin-like sequences at distance, are accompanied through yet unknown steps by methylation of lysine 9 of histone H3 by the modifier of PEV Su(var)3-9 (Schotta et al., 2002). This would lead to the recruitment of HP1, which in turn recruits Su(var)3-7. Then compaction of chromatin by Su(var)3-7 imposes silence.

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