Mutations in the *Drosophila melanogaster* Gene Encoding S-adenosylmethionine Suppress Position-Effect Variegation

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ABSTRACT

In *Drosophila melanogaster,* the study of *trans*-acting modifier mutations of position-effect variegation and *Polycomb* group (Pc-G) genes have been useful tools to investigate genes involved in chromatin structure. We have cloned a modifier gene, *Suppressor of zeste 5* (*Su(z)5*), which encodes S-adenosylmethionine synthetase, and we present here molecular results and data concerning its expression in mutants and genetic interactions. The mutant alleles *Su(z)5, l(2)R23* and *l(2)M6* show suppression of w^{m4} and also of two *white* mutants induced by *roo* element insertions in the regulatory region *i.e.*, w^{is} (in combination with z^1) and w^{sp1}. Two of the *Su(z)5* alleles, as well as a deletion of the gene, also act as enhancers of *Polycomb* by increasing the size of sex combs on midleg. The results suggest that *Su(z)5* is connected with regulation of chromatin structure. The enzyme S-adenosylmethionine synthetase is involved in the synthesis of S-adenosylmethionine, a methyl group donor and also, after decarboxylation, a propylamino group donor in the bio-synthesis of polyamines. Our results from HPLC analysis show that in ovaries from heterozygous *Su(z)5* mutants the content of spermine is significantly reduced. Results presented here suggest that polyamines are an important molecule class in the regulation of chromatin structure.

ONE approach to understanding the control of differential gene expression has been to study *trans*-acting regulatory factors in *Drosophila melanogaster.* This is most often done through dominant mutations that alter the expression of a given genetic model system. Two genetic model systems, the *zeste-white* interaction and position-effect variegation, have easily scored phenotypic changes and have consequently been subject to extensive studies. Recently, the effects of the Pc-G (*Polycomb* group) genes on the regulation of homeotic gene expression have also become a widely used assay to study gene regulation. Results indicate that the role in gene regulation of these three systems may be caused by a common molecular mechanism, namely changes of chromatin structure (reviewed by PIRROTTA 1991; ORLANDO and PARO 1995).

The basis of the *zeste-white* system is a particular mutant allele of *zeste,* namely z^1. This mutant represses the eye-specific expression of the *white* gene, resulting in a yellow eye color instead of the wild-type red eye color (GANS 1953; BINGHAM and ZACHAR 1985). This repression requires two copies of the *white* gene in close proximity, which occurs either by homologous chromosome pairing or by tandem duplications (JACK and JUDD 1979; reviewed by WU and GOLDBERG 1989; PIRROTTA 1990). The product of the *zeste* gene is a nuclear DNA-binding protein found associated with over 60 specific loci on the polytene chromosomes including the major homeotic complexes (PIRROTTA *et al.* 1988; RASTELLI *et al.* 1993). A multimerization of the Zeste protein is thought to be required for chromosome pairing. The $zeste^1$ mutation has been shown to cause formation of larger Zeste aggregates than the wild-type gene and the repression of *white* by $zeste^1$ is correlated with the hyper-aggregation of the *zeste* gene product (CHEN and PIRROTTA 1993). In the early 1970s, the first mutagenesis screen for dominant suppressors of z^1 repression of a specific *white* allele, w^{is}, was presented (KALISCH and RASMUSON 1974). In this screen seven dominant modifiers of z^1 w^{is} were isolated. Of these, *Suppressor of zeste 2* and *Enhancer of zeste 1* have been extensively studied. *Su(z)2* suppresses the z^1-mediated repression of *white* not only in z^1 w^{is} males but also in z^1 w^+ females, suppressing the yellow eye color to wild-type red. *E(z)1* gives the opposite effect; in combination with z^1 w^{is} males or z^1 w^+ females, *E(z)1* enhances the repression and gives a light yellow eye color. Both these genes have been cloned (BRUNK *et al.* 1991; JONES and GELBART 1993). *E(z)* is considered to be a member of the *Pc* group (PHILLIPS and SHEARN 1990), while *Su(z)2* is at least functionally related to some of the members in the Pc-G on the basis of its interaction with *Posterior sex combs* (*Psc*) and *Sex combs on midleg* (*Scm*) (ADLER *et al.* 1989; WU *et al.* 1989). The characterization of the two genes have led to a mechanistic connection between the $zeste^1$-*white* interaction and the regulation of genes by the Pc-G gene products. It has been shown by RASTELLI *et al.* (1993) that the Psc and Su(z)2 proteins bind to ~80–

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90 locations on salivary gland polytene chromosomes, and a comparison of these locations with the chromosomal binding sites for Zeste, Polycomb and Polyhomeotic shows that the proteins colocalize at a large number of sites, suggesting that they act cooperatively in regulation of target genes.

The Pc-G genes encode proteins that maintain a repressed state of the homeotic segment identity genes in the ANT-C and BX-C gene complexes (FRANKE *et al.* 1992). The correct expression of Pc-G genes together with the *trithorax* group genes are of vital importance for the correct maintenance of the determined state of cells, and thus necessary for the proper development of an organized body plan. The mechanism for this maintenance of a determined state of gene expression has been proposed to be the formation of closed heterochromatin-like structures (PARO and HOGNESS 1991). The main arguments for this are molecular similarities and shared physiological properties between Pc-G proteins and modifiers of position-effect variegation (reviewed by ORLANDO and PARO 1995).

Position-effect variegation (PEV) was first characterized by MULLER (1930) as the variable, but heritable, repression of euchromatic genes when rearrangements juxtapose them to heterochromatin. For example, when an inversion places a *white*$^{+}$ gene next to heterochromatin, the inactivation is seen as a variegating eye with pigmented and nonpigmented clones of ommatidia (for reviews, see HENIKOFF 1990; REUTER and SPIERER 1992). The most commonly used model system for PEV is the w^{m4} inversion described by MULLER (1930). Different mechanisms have been proposed to explain the PEV phenomenon, where the model of a multimeric assembly of chromatin, and its impact on transcription activity is fundamental. Lately however, strong evidence for more complex mechanisms has been published, including nuclear compartmentalization and physical alterations of DNA (reviewed by KARPEN 1994). Correlation between gene regulation caused by the Pc-G genes and by PEV was indicated when a 52-amino acid-long chromo domain was shown to be present in both the Polycomb protein and the heterochromatin binding protein 1 (HP1) (PARO and HOGNESS 1991). The HP1 protein is encoded by the *Su(var)205* gene and mutants act as dominant modifiers of PEV. Further correlation between the two systems has been demonstrated by the fact that a regulatory region from the *ph* gene, which responds to Pc-G repression, can induce variegation of an adjacent *white* gene (FAUVARQUE and DURA 1993).

The results reported here seem to link these three model systems together. We have studied the *Su(z)5* gene and found that *Su(z)5* mutations, apart from being dominant suppressors of z^{1} w^{is}, also act as dominant enhancers of *Pc* and as suppressors of PEV. In this paper we present evidence that the mutant *Su(z)5* is caused by changes in the gene encoding the enzyme S-adenosylmethionine synthetase. We also argue that the dominant suppressor effect on z^{1} w^{is} and on w^{m4}, together with the enhancer effect on *Pc*, is likely to be caused by a decrease in spermine concentration and that polyamines are important molecules in the assembly of higher order chromatin structure.

MATERIALS AND METHODS

Drosophila stocks and culture: For a description of mutants used see LINDSLEY and GRELL (1968) and LINDSLEY and ZIMM (1992). Stocks were kindly provided by C. CAGGESE (*l(2)M, l(2)R, l(2)PM* and *Df(2L)PM* stocks), M. M. GREEN (*Df(2L)(net, lgl)78:30* and z^{1} w^{sp2}), B. MECHLER (*Df(2L)l(2)gl net3*) and the Umeå Drosophila Stock Center. All eye color comparisons were made on parallel cultures of equal age. All crosses were repeated at least twice. Crosses were made in vials with potatomash-yeast-agar medium at temperatures indicated in the text. We have found that several balancer chromosomes contain modifiers of PEV that can interfere with the experiments. Therefore, before examining the suppression of PEV, the mutants were first rebalanced to *Df(2L)S2* (2L:21C6-D1;22A6-B1), to homogenize the second chromosome and to use the *Df(2L)S2* chromosome as a control. The balanced strains *M//Df(2L)S2* where *M* stands for *Su(z)5, l(2)M6, l(2)R23* or *Df(2L)PM44* were checked every generation to prevent propagation of crossovers.

DNA isolation and Southern analysis: Genomic DNA was prepared according to the protocols described in SAMBROOK *et al.* (1989). DNA was cut with restriction enzymes indicated in Figure 1, separated on 0.8% agarose gels, transferred to GeneScreenPlus filter membranes (DuPont-NEN Research Products Inc.) and hybridized according to the instructions of the manufacturer.

poly(A)$^{+}$ RNA extraction: poly(A)$^{+}$ RNA was extracted using Dynal biomagnetic separation system. Ovaries or testes were frozen in ethanol/CO_2-ice bath. The frozen tissue was homogenized in 0.1 M Tris-HCl (pH 8.0), 0.5 M LiCl, 10 mM EDTA, 1% SDS, 5 mM dithiothreitol (DTT). After this step the instructions from the manufacturer, Dynal, were followed.

Reverse Northern analysis: poly(A)$^{+}$ RNA from pupae was labeled radioactively as described in SAMBROOK *et al.* (1989) and used as a probe in hybridizations to overlapping genomic DNA fragments from the 21A-B region (cloned in λ) blotted onto GeneScreenPlus filters (DuPont-NEN).

Northern analysis: Approximately 0.3–1.0 μg of poly(A)$^{+}$ RNA from ovaries were separated on an 1.0% formaldehyde-agarose gel as described by HANSSON and LAMBERTSSON (1983) and blotted onto GeneScreenPlus filters (DuPont-NEN) using VacuGene Vacuum Blotting System (Pharmacia LKB Biotechnology AB). The filters were prehybridized, hybridized and washed according to the instructions for GeneScreenPlus filters. The cDNA clone #10 (LARSSON and RASMUSON-LESTANDER 1994) and α-tubulin (kindly provided by A. LAMBERTSSON) labeled by the random priming technique were used as probes.

HPLC analysis: To determine the content of polyamines, ovaries were sonicated in 0.2 M perchloric acid and centrifuged. The supernatant was analyzed for spermine and spermidine content using the reverse phase HPLC method described by SEILER and KNÖDGEN (1985). Polyamines were determined by separation of the ion pairs formed with 1-octanesulfonic acid on a reversed-phase column (Kromasil KR 100-5C18; Eka Nobel; 15 cm × 4.6 mm inside diameter). For each strain double samples were analyzed. Each sample contained 100 ovaries from 5-day-old females (25°) and two aliquots were run separately.

Enzyme activity assay: Proteins were extracted by grinding 40 ovaries in 400 μl of 2× extraction buffer [100 mM Tris (pH 7.5), 2 mM EDTA, 20% glycerol, 20 mM β-mercaptoethanol, 1 mM DTT). After centrifugation at 13,000 rpm for 5 min, the supernatant was collected and protein concentration was determined (BRADFORD 1976) using the Bio-Rad Protein Assay kit. The AdoMet synthetase activity assay was modified from MUDD *et al.* (1965). One hundred micrograms protein extract was incubated in 250 μl reaction buffer [100 mM Tris (pH 8.0), 30 mM $MgSO_4$, 10 mM KCl, 7.5 μCi [^{35}S]-methionine (Amersham) and 10 mM ATP] at 37° for 30 min. Control reactions were without ATP. The reactions were stopped by adding 2 ml of ice-cold water. Reactions were loaded on Dowex AG 50W-X2 cation exchange columns (NH_4^+ form) and columns were washed with 20 ml cold water. The adsorbed S-adenosylmethionine was eluted with 5.0 ml of NH_4OH (29.1%). The collected samples were measured by scintillation spectrometry. Protein extractions and activity assays were repeated eight times for each strain.

Sequencing: Fragments from the genomic λ-clones y36-1 and y34-3 (kindly provided by H.-P. LERCH) were subcloned into pUC19 vector using standard techniques. DNA for sequencing was prepared by the Wizard Mini Prep DNA Purification System (Promega Corp.) and sequenced with the dideoxy chain termination technique (SANGER *et al.* 1977) using the Promega Taq Track sequencing kit and [^{35}S]dATP (Amersham) following instructions from the supplier. Forward and reverse primers for pUC/M13 vector were used as well as internal primers (Symbicom). Samples were run at 2.5, 6, and 9 hr on a 5% polyacrylamide gel at constant power (75 W).

RESULTS

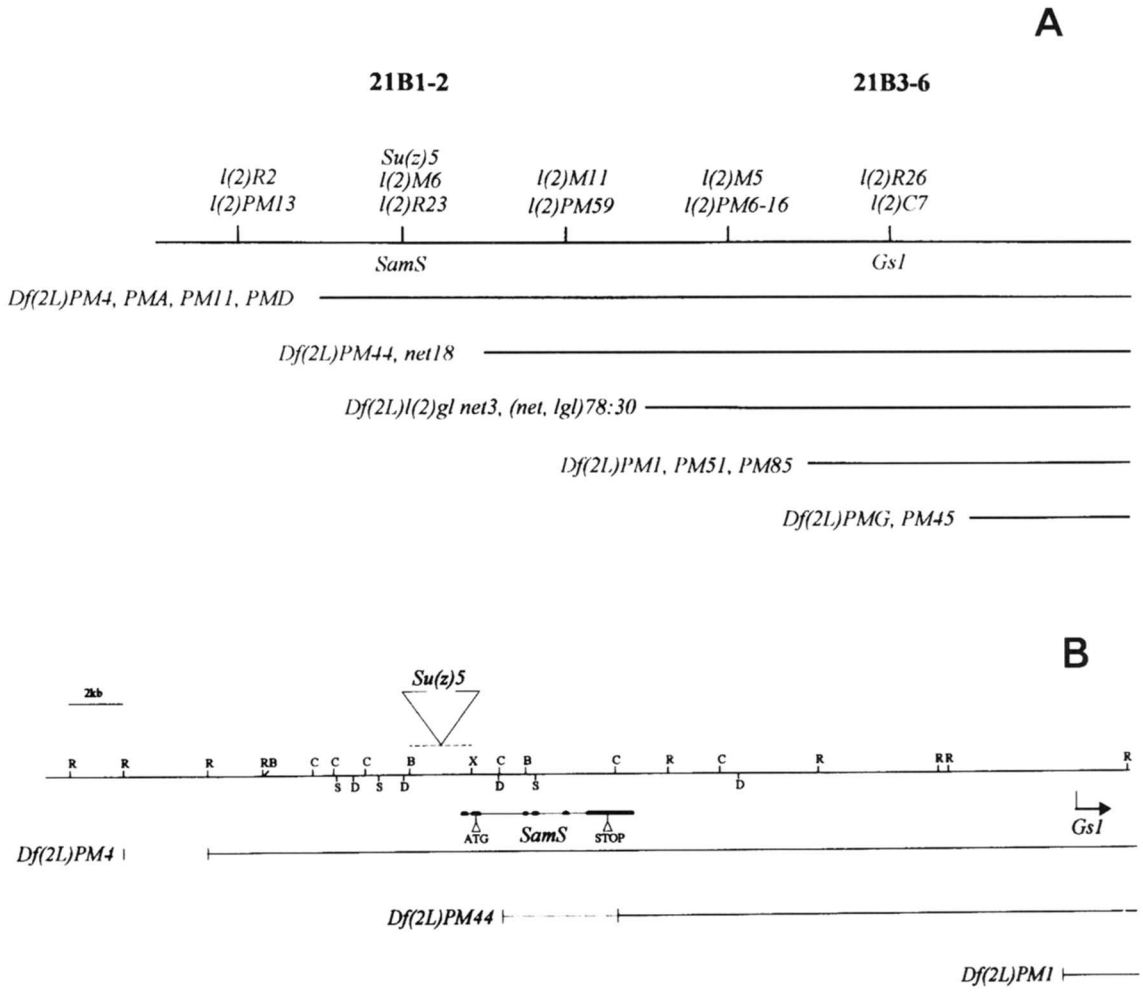
Phenotypes of *Su(z)5* mutants: The *Suppressor of zeste 5* mutant was isolated in the same EMS mutagenesis screen as the well-studied *E(z)* and *Su(z)2* mutants (KALISCH and RASMUSON 1974). PERSSON (1976) described *Su(z)5* as a dominant factor on chromosome *2* (2-0.0) with pleiotropic effects and as a homozygous embryonic lethal. In addition to the suppressor effect on z^1 w^{is}, he also reported that the developmental time was increased by 3 days in heterozygous flies. These flies also showed the characteristic bristle phenotype of *Minute* mutants, and females were sterile when reared above 18°. This mutant has therefore sometimes been denoted *M(2)21A-B* (PERSSON 1976; LINDSLEY and ZIMM 1992). The mutant was also described to be unstable, and revertants lacked both the Minute and the suppressor phenotypes as well as the lethality located to 2-0.0 (PERSSON 1976).

Our copy of the *Su(z)5* mutant strain lacks the developmental delay, the Minute characteristics and the female temperature sensitive sterility but retains the Suppressor of zeste effect and the embryonic lethality. Therefore, we prefer the name *Su(z)5* instead of *M(21)A-B*. We have not observed any instability in our *Su(z)5* mutant stock.

Localization of *Su(z)5*: The gene *Su(z)5* was previously localized by recombination to 0.0 on 2L (PERSSON 1976). To obtain more alleles of *Su(z)5* and to more accurately locate the gene, we performed complementation analysis and deletion mapping using known mutants and deficiencies in the 21A-B region (Figure 1A). We were able to separate four complementation groups of recessive lethals distal to the *Gs1* (*Glutamine synthetase 1*) gene in 2L:21B3-6. This map has been verified both by us and C. CAGGESE (CAGGESE *et al.* 1988; C. CAGGESE, personal communication). The lethality of *Su(z)5* was not complemented by *l(2)R23*, *l(2)M6* or by *l(2)PM13* and *l(2)R2*, which belong to a different complementation group. Upon testing all mutations and deficiencies listed in Figure 1A for suppression of z^1 w^{is} (results not shown) only *l(2)M6*, *l(2)R23* and *Su(z)5* were dominant suppressors of z^1 w^{is}. These results showed that the suppressor effect is located in the complementation group of *l(2)M6* and *l(2)R23*. Deficiencies uncovering this locus did not suppress z^1 w^{is}.

Genetic interactions: To see whether the suppression observed was exclusively directed toward z^1 w^{is} interaction we combined several *white*-alleles with *Su(z)5*, *l(2)M6*, *l(2)R23* and two deficiencies uncovering this locus. The results from these experiments are shown in Tables 1 and 2. The orange brown color of z^1 w^{is} males is suppressed to reddish brown but not fully wild type (Figure 2) by *Su(z)5* and *l(2)M6* and to a lesser extent by *l(2)R23*. The suppression of z^1 w^{is} is seen both at 25° and at 20° with *Su(z)5* and *l(2)M6* while the suppression is discernible at 25° with *l(2)R23* (Table 1). We found that *Su(z)5* and *l(2)M6* are strong suppressors of w^{m4} (*i.e.*, Su(PEV)) (Figure 2) and that *l(2)R23* also suppresses w^{m4}, but the effect is less pronounced. The deletions *Df(2L)net18* and *Df(2L)PM44*, however, did not suppress w^{m4} (Table 1). In addition to z^1 w^{is} we found that w^{sp1} was suppressed by *Su(z)5* in males with a z^+ background (Table 2). Note that both w^{is} and w^{sp1} are induced by an insertion of a *roo* element in the regulatory region of *white*. In w^{sp1} the *roo* element is inserted at +4922 (O'HARE *et al.* 1984), and in w^{is} the insertion is at +4707 (J. LARSSON, unpublished results). This indicates that *roo* may be a target for the suppression function. *Su(z)5* also suppresses z^1 w^{+uz}, induced by *FB-NOF* at ~ −5000 (Å. RASMUSON-LESTANDER, unpublished results) and z^1 *Dp(1;1)* $w^{+r61e19}$. No suppression was detected with the *Df(2L)PM44* allele in any of the tested *zeste-white* constellations.

Since the previously cloned modifiers of z^1 w^{is} interaction were classified as Pc-G- or Pc-G-like genes we also wanted to test whether *Su(z)5* acts as a modifier of *Pc*. Results from these experiments are shown in Table 3. We crossed Pc^{11}//*TM3* females with *M*//*CyO* [*P ftz:lacZ*] males. In the male offspring from this cross we counted the number of midlegs with sex combs and the number of teeth in these combs. The *M*//+; Pc^{11}//+ males were compared to the *CyO*//+; Pc^{11}/+ males from the same cross. We found that the number of midlegs with sex combs and the number of teeth per sex comb were significantly increased in *Su(z)5* and *l(2)M6* compared to controls, whereas with the null-allele, *Df(2L)PM44*, only the number of teeth was significantly increased.



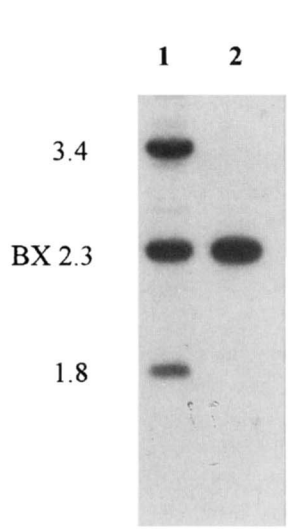


FIGURE 1.—(A) Complementation map of the 2L:21A-B6 region. (B) Restriction map of the region including the *SamS* gene. Deficiency breakpoints are indicated. The large arrowhead shows the insertion of at least 2.9 kb in the *Su(z)5* mutant. The *SamS* gene consists of six exons: exon 1, 134 bp; 2, 222 bp; 3, 77 bp; 4, 126 bp; 5, 108 bp; 6, 1694 bp The λ-clone y36-1 covers DNA from 10 kb upstream of the transcription start to 8 kb downstream. The transcription startpoint of the *Gs1* (*Glutamine synthetase 1*) gene is indicated by an arrow. According to C. CAGGESE (personal communication) *l(2)R2* and *l(2)PM13* are alleles of *l(2)gl*, which resides ~90 kb distal to *SamS*. B, *Bam*HI; C, *Sac*I; D, *Hin*dIII; R, *Eco*RI; S, *Sal*I; X, *Xho*I. Distal to the R site 7.5 kb upstream of the transcription start only *Eco*RI sites are indicated. (C) Southern blot analysis. Genomic DNA from *Su(z)5//CyO* (1) and *l(2)M6//CyO* (2) were digested with *Bam*HI and *Xho*I. The *Bam*HI-*Xho*I 2.3-kb fragment containing the transcription start (see Figure 1B) was used as a probe. The 2.3-kb band in lane 1 derives from the *CyO* chromosome.

TABLE 1

Analysis of suppression of *Su(z)5* alleles on *z w^{is}* and *In(1)w^{m4}*

Allele	*z w^{is}*		*In(1)w^{m4}*
	25°	20°	20°
Su(z)5	Weak	Weak	Strong
l(2)M6	Weak	Weak	Strong
l(2)R23	Very weak	Weak	Weak
Df(2)PM44	None	None	None
Df(2)net18	None	None	None

No differences from control were found with *l(2)R23*. These results show that the *Su(z)5* and *l(2)M6* alleles act as dominant enhancers of Pc^{11} and that the null-mutation *Df(2L)PM44* also at least partly enhances Pc^{11} (Table 3).

Cloning: We verified the genetical analysis of breakpoints through genomic Southern blot analyses of the deficiency stocks *Df(2L)PM4, Df(2L)PM44,* and *Df(2L)PM1* (Figure 1B). From this we could locate the *Su(z)5* gene to a specific λ-clone, y36-1, from a chromosome-walk done by H.-P. LERCH within this region. To locate the transcribed regions within this clone, radioactively labeled first strand cDNA, made from pupal poly(A)$^+$ RNA, was hybridized to restriction fragments of the λ-clone y36-1. Results from this experiment showed that the 15-kb *Eco*RI fragment within this clone was transcribed (results not shown). We then used this 15-kb *Eco*RI fragment as a probe in a cDNA library screen. Out of 400,000 clones, nine positive were isolated and all contained the transcription unit for the Drosophila gene encoding S-adenosylmethionine synthetase (LARSSON and RASMUSON-LESTANDER 1994). These results indicate that the gene encoding S-adenosylmethionine synthetase is a candidate gene for *Su(z)5*. To determine the structure of the gene, we sequenced genomic DNA. We found that the gene consists of six exons as shown in Figure 1B. Southern blot analysis showed that the *Su(z)5* allele is caused by an insertion of at least 2.9 kb within the 2.3-kb *Bam*HI-*Xho*I fragment containing the *Su(z)5* transcription start (Figure 1C). An *in situ* hybridization to polytene chromosomes, using the 3.6-kb *Hin*dIII fragment containing the transcription start point as a probe, located the gene to 2L:21B1-2 (results not shown).

Expression: It has been shown that the highest amount of transcription of the S-adenosylmethionine synthetase gene is found during the adult stage, and in females a high level of transcript is found in ovaries (LARSSON and RASMUSON-LESTANDER 1994). To find out if transcription of the gene encoding S-adenosylmethionine synthetase is affected in the different *Su(z)5* alleles, we compared expression levels in wild-type ovaries and ovaries from mutant heterozygotes. poly(A)$^+$ RNA from 15 ovaries per strain were separated on an agarose gel and hybridized with the cDNA clone #10 as probe. This result shows that all mutants produce mRNA of the same size as wild-type females. On the other hand there is a reduction of transcript in the mutants *Su(z)5* and *Df(2L)PM44* compared to wild type. The *l(2)R23* and *l(2)M6* alleles have mRNA levels comparable to wild type (Figure 3).

The expression was further analyzed using an enzyme activity assay measuring the S-adenosylmethionine synthetase activity in crude protein extracts. The highest

TABLE 2

Analysis of suppression effects of the *Su(z)5* allele on various *white* alleles

Allele	*white* locus lesion	Suppression (20°)	Reference
z w^{is}	*roo*-element in +4707	Weak	J. LARSSON (unpublished results)
w^{sp1}	*roo*-element in +4922	Weak	O'HARE *et al.* (1984)
z w^{+uz}	*FB-NOF* in ca. −5000	Weak	Å. RASMUSON-LESTANDER (unpublished results)
z Dp(1;1)$w^{+r61e19}$	Duplication + *BEL*	Weak	GOLDBERG *et al.* (1983)
z w^{sp2}	Deletion in 5′ regulation region	None	LINDSLEY and ZIMM (1992)
z w^{sp55}	*mdg3* in 5′ untranslated leade	None	CSINK *et al.* (1994)
z w^{negro}	Unknown	None	
z w^{zm}	*BEL* in intron 1, +3430	None	PETERSON *et al.* (1994)
z w^{zmzrh}	Unknown	None	
z w^{zmzw}	Unknown	None	
z w^{ch}	*pogo* insertion in *Doc* of w^1	None	LINDSLEY and ZIMM (1992)
z w^{ci}	*copia* in intron 2	None	LINDSLEY and ZIMM (1992)
w^{ft}	*copia* in intron 2	None	LINDSLEY and ZIMM (1992)
w^{a2}	Point	None	ZACHAR and BINGHAM (1982)
w^{bf2}	*roo*-element in 5′ untranslated leader	None	CSINK *et al.* (1994)
w^{bf61d5}	Unknown	None	
w^{bf}	*roo*-element in intron 4	None	ZACHAR and BINGHAM (1982)
w^{h}	*roo*-element in *Doc* of w^1	None	LINDSLEY and ZIMM (1992)
w^{co}	Unknown	None	
w^{m4}	Inversion	Strong	LINDSLEY and ZIMM (1992)

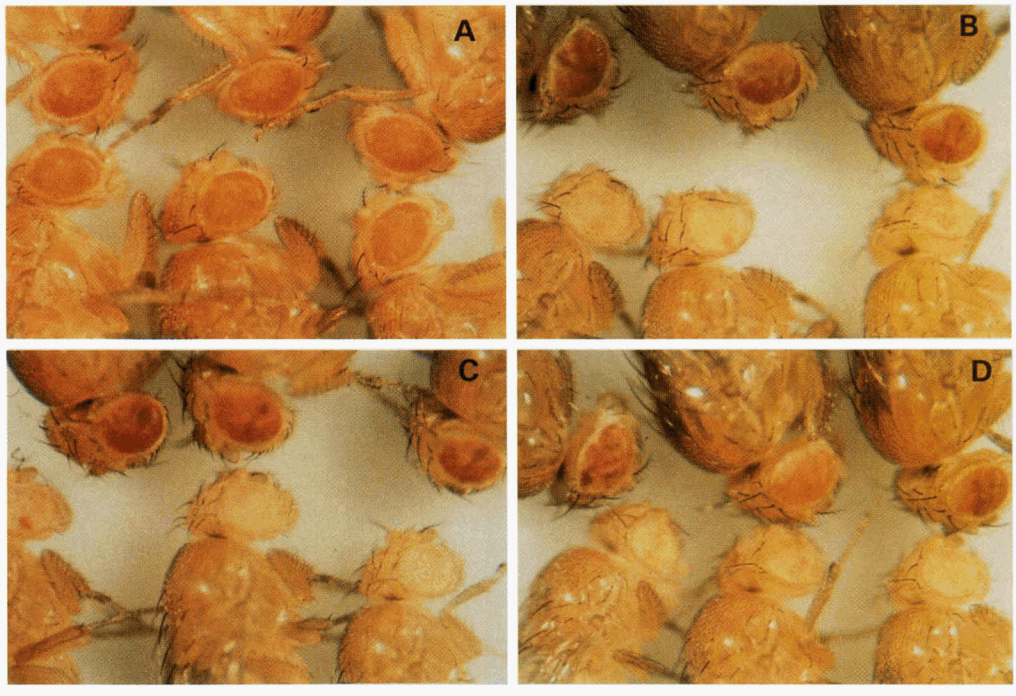


FIGURE 2.—(A) Typical phenotypes of z^1 w^{is}; *Su(z)5* (top) and z^1 w^{is}; *CyO* (bottom). (B) Phenotypes of w^{m4}; *Su(z)5* (top) and w^{m4}; *Df(2L)S2* (bottom). (C) Phenotypes of w^{m4}; *l(2)M6* (top) and w^{m4}; *Df(2L)S2* (bottom). (D) Phenotypes of w^{m4}; *l(2)R23* and w^{m4}; *Df(2L)S2*. Crosses were done at 20° and comparisons were made with flies of equal age. In w^{m4} crosses, *Df(2L)S2* was used as control (see MATERIALS AND METHODS).

activity was found in ovaries, and extracts were therefore prepared from both wild-type and heterozygous mutant ovaries. Results show that the enzyme activity is significantly reduced in the four mutants compared to wild type (Figure 4).

Polyamines: S-adenosylmethionine synthetase catalyzes the reaction that gives S-adenosylmethionine (AdoMet) as product. AdoMet is the methyl donor of all methylation reactions, except for the methylation of methionine itself. It also serves as a propylamino donor, after decarboxylation, in the polyamine synthesis pathway. To study if there is a change in polyamine content in the *Su(z)5* mutants, we measured the polyamine levels in ovaries from wild-type flies and heterozygous mutants using an HPLC technique. The results from these experiments are given in Figure 5 and show a reduction in the spermidine content in *l(2)M6* ovaries and in the spermine content in *l(2)M6, Su(z)5* and *Df(2L)PM44* ovaries.

DISCUSSION

The deficiency mapping shows conclusively that there is only one complementation group defined by the breakpoints of *Df(2L)PM44* and *Df(2L)PMA*. The Northern blot analysis revealed only a single transcription unit within this region. We therefore conclude that the phenotypic changes seen in *Su(z)5* mutants are

TABLE 3

Interaction of *Su(z)5* alleles with Pc^{11}

Allele	% midlegs with sex-comb[a]	χ^2 value	Mean no. of teeth per sex-comb[b]	*t*-test
Su(z)5	58.6 (198)	21.5***	2.96 ± 1.99	5.04***
CyO	35.3 (198)		1.89 ± 0.88	
l(2)M6	40.4 (198)	9.9**	2.63 ± 1.22	5.03***
CyO	25.5 (196)		1.72 ± 0.83	
l(2)R23	21.8 (188)	0.82	1.83 ± 0.79	0.02
CyO	25.8 (186)		1.83 ± 0.85	
Df(2L)PM44	43.0 (186)	0.374	2.96 ± 1.82	3.38***
CyO	39.9 (188)		2.16 ± 1.05	

Levels of significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.
[a] Total numbers of legs in parentheses.
[b] Values are means ± SD.

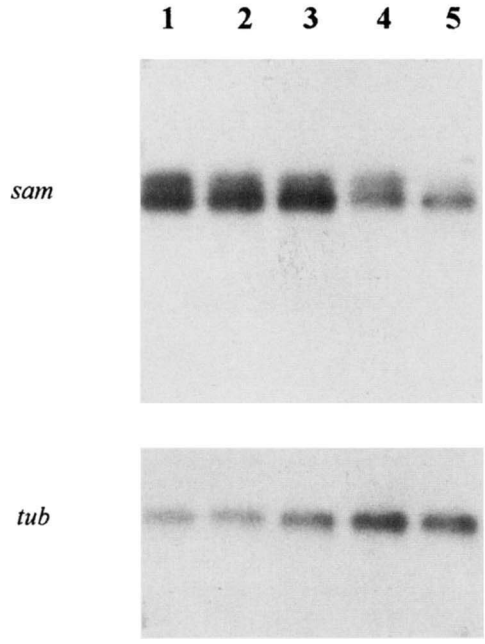


FIGURE 3.—Northern blot of poly(A)$^+$ RNA from ovaries hybridized with *sam* (cDNA clone #10) and with α-tubulin as control. Lane 1, wild type; 2, *l(2)M6//Cy;* 3, *l(2)R23//SM6a;* 4, *Su(z)5//CyO;* 5, *Df(2L)PM44//SM6a.*

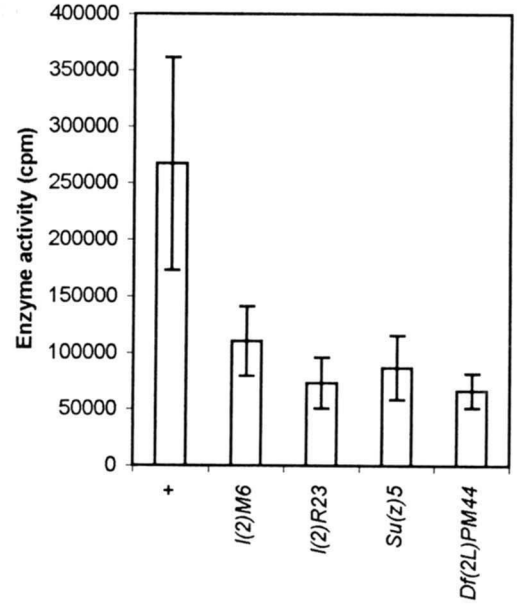


FIGURE 4.—S-adenosylmethionine synthetase activity. The decreases in enzyme activity seen in the heterozygous mutants are all statistically significant (*t*-test, $P < 0.001$). + , wild type.

caused by decreased amounts or lowered activity of the S-adenosylmethionine synthetase enzyme.

Expression in mutants: The decrease of S-adenosylmethionine synthetase enzyme activity in the three *Su(z)5* alleles, *Su(z)5, l(2)M6* and *l(2)R23,* together with the fact that there is neither a reduction in the amounts of transcript in *l(2)M6* and *l(2)R23* nor a change in transcript size, suggest that the two latter alleles represent point mutations in the gene encoding S-adenosylmethionine synthetase. These mutations lead to full length mRNAs that are either nontranslated or give less/nonfunctional proteins. Results from Southern blot analysis indicate that *Su(z)5* is caused by an insertion of at least 2.9 kb in the regulatory region of *Su(z)5.* This regulatory mutation prevents transcription, which is observed as lower amounts of transcript, lower enzyme activity and lower spermine content. However, there remains an enigma; why does the null mutant *Df(2L)PM44,* in spite of showing a reduction in enzyme activity, not show the suppressor effects? Both *Su(z)5* and *Df(2L)PM44* show reduced amounts of transcript compared to *l(2)M6* and *l(2)R23,* but only *Su(z)5, l(2)M6* and *l(2)R23* exhibit suppressor phenotypes. At least two possible explanations exist to account for this. First, since *Df(2L)PM44* deletes the tip of chromosome 2L, that is >100 kb, it is possible that this deficiency deletes distal regions that counteract the suppressor effect. Alternatively, a differential control of the functional *Su(z)5*$^+$ gene in the balancer chromosome between the mutants could exist. In the three suppressor-mutant strains, the transcriptional control of *Su(z)5*$^+$ in the balancer chromosome might be kept stringent due to the presence of two functional *cis*-regulatory regions, where *Df(2L)PM44* has only one.

Genetic interactions: For the repression of *white* by z^1 to occur, two *white*$^+$ copies (or at least two Zeste binding regions) have to be paired. In yellow eyed z^1 females, this is thought to be achieved by somatic pairing of the two *X* chromosomes. The single *white* gene in z^1 w^{is} males is also repressed, giving an orange eye color. Since the *X* chromosome in males cannot pair to a homologue, this repression is achieved either by an alteration of the accessibility of the Zeste binding sites in the *white* promoter region caused by *roo* or by a more unspecific pairing between the w^{is} locus on the *X* chromosome and some other region on *X, A* or the *Y* chromosome, again caused by the *roo* insertion. The latter alternative has been proposed for a rearranged w^{is} chromosome, *In(1)*w^{is}, where pairing between the *X* and the *Y* chromosome was shown to be essential for repression by z^1. This inversion places the w^{is} copy close to centromeric heterochromatin, and it was shown that deletions of the homologous region in the *Y* chromosome (*i.e.,* the *bobbed* locus) suppressed the z^1 *In(1)*w^{is} phenotype (RASMUSON-LESTANDER *et al.* 1993). The idea that changes in chromatin structure can be induced by proximity to other sequences in *trans* was proposed by ASHBURNER (1977). The propagation of heterochromatin across paired homologues has also been proposed to explain the dominant position effects at the *bw* locus (HENIKOFF and DREESEN 1989). The *trans*-inactivation that occurs in the bw^D locus has been studied in detail by TALBERT *et al.* (1994). In addition to the effect of the homologous genes associated by somatic pairing, the effects of an inversion-loop that places the *brown* gene into a heterochromatic region

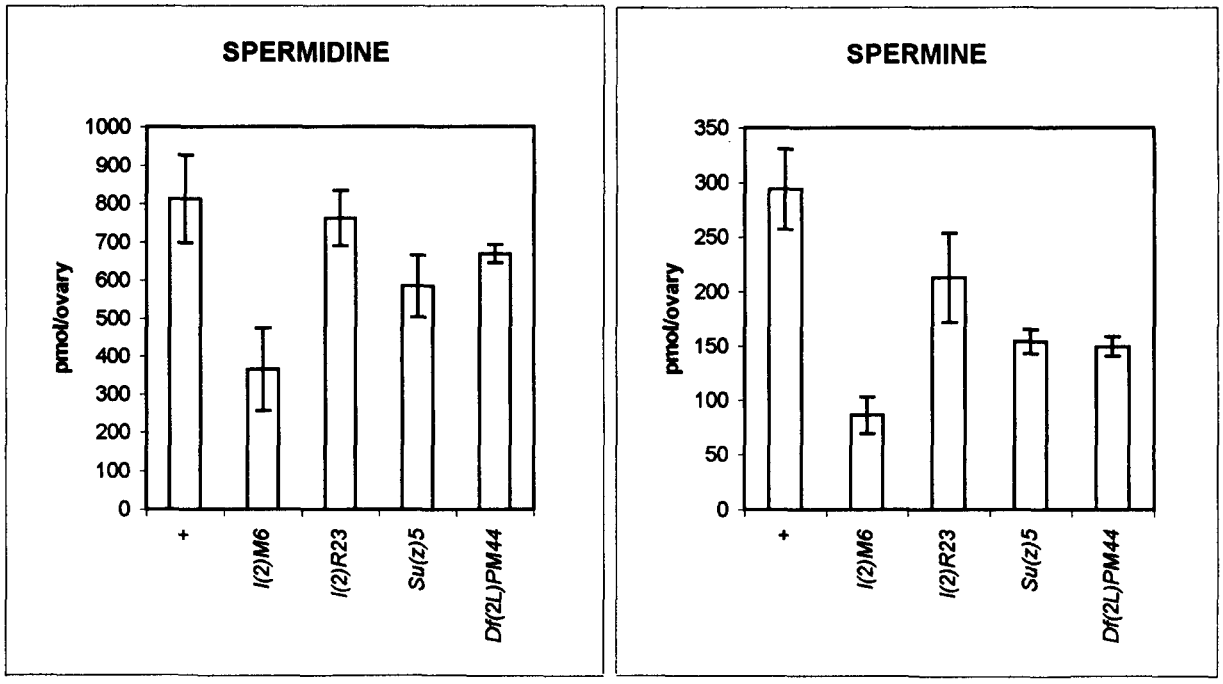


FIGURE 5.—Polyamine content per ovary. The decrease of spermidine in *l(2)M6* heterozygotes compared to wild type is statistically significant ($P < 0.05$). The decrease of spermine is statistically significant in *Su(z)5, Df(2L)PM44* ($P < 0.05$) and *l(2)M6* ($P < 0.01$). The results are based on duplicate testing. +, wild type.

indicate nuclear positioning as a major cause for PEV. As shown, *Su(z)5* suppresses *roo*-induced mutations and PEV. This suggests that the reason why z^1 represses *white* when juxtaposed to *roo* (*i.e.*, z^1 w^{is} males) could be an alteration of chromatin structure, induced by pairing that simultaneously facilitates the assembly of the multimeric Zeste complex. *Su(z)5* mutant alleles enhance the effect of Pc^{11}, which is expected considering that both *Su(z)5* and Pc^{11} mutants decrease the degree of DNA condensation. This could also explain why *E(z)* and *Su(z)2* that also were isolated as z^1 w^{is} modifiers both interact with *Polycomb*.

Our results show that the reason for the mutant phenotypes is most probably a decrease of the molecule S-adenosylmethionine (AdoMet). This molecule is involved in methylation reactions and in the synthesis of polyamines, which allows us to speculate on a model for the modifying mechanism of *Su(z)5* mutants.

Models for suppression: Three alternative models for the suppressor effects of S-adenosylmethionine synthetase can be hypothesized: (1) polyamines are components in multimeric complexes, (2) polyamines interact directly with DNA, and (3) AdoMet methylates DNA. In model 1, polyamines may be an important class of molecules for building up the protein complexes, consisting of, *e.g.*, *zeste*, Pc-G or trx-G gene products, and that they are needed for determining the expression status of a gene in a special cell type. That is, polyamine molecules are needed to achieve correct multimeric protein tertiary structures.

In model 2, polyamines could bind directly to DNA and thus affect the packaging of the DNA. It has been suggested that the acetylation of polyamines and histones act synergistically to modulate chromatin structure and that polyamines have a role in the regulation of the nucleosome structure (reviewed by MATTHEWS 1993). Spermine has also been shown to stabilize unusual DNA structures such as A DNA and Z DNA, and to favour these structures over B DNA (reviewed by FEUERSTEIN *et al.* 1991). Spermine has also been predicted to cause sequence specific bends of DNA and this may have important implications for the regulation of genomic tertiary structures.

Even though polyamines are often used in experiments designed to examine chromatin structure, the role and function of these molecules have until lately been somewhat neglected (MATTHEWS 1993). Our results support the view that polyamines have an important role in modulating chromatin structure, and they also indicate that the concentrations of polyamines in certain stages of development are crucial for correct expression of genes that are influenced by chromatin structure.

Regarding model 3, methylation of DNA seems to play an important role during development in regulating gene expression in vertebrates. DNA methylation appears to inhibit transcription by preventing transcription factors from binding and by altering the chromatin structure (reviewed by EDEN and CEDAR 1994). In *D. melanogaster*, however, little or no (<1 per 10 kb) 5-methylcytosine (5mC) and 6-methyladenine (6mA) has been found (BIRD and TAGGART 1980; URIELI-SHOVAL

et al. 1982), although ACHWAL *et al.* (1983) claim that they have been able to detect 5mC, 6mA and 7-methylguanine by an immunochemical method. In contrast to vertebrate DNA, there is neither a deficit of CpG doublets in Drosophila nor any evidence for methylation of CCGG sites, detectable by restriction enzymes (ASHBURNER 1989). Taken together, even if DNA methylation is a mechanism that implements changes in the chromatin structure and thus regulates transcription, the extremely low levels of DNA methylation that might exist in Drosophila are not likely to account for the results we have obtained in the *zeste-white,* the PEV and the Pc-G assays.

The HPLC results show more severe deficits of spermine than of spermidine. This is expected if the decrease in enzyme activity affects the synthesis of polyamines since two molecules of decarboxylated AdoMet are needed for the synthesis of spermine compared to one group in the synthesis of spermidine. Since spermine has been shown to bind to DNA, preferably to the major groove (reviewed by FEUERSTEIN *et al.* 1991) and since *Su(z)5* mutants have a deficit of spermine, we propose that it is the decrease in spermine concentration that gives the modifying effects obtained.

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