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Characterization of the *grappa* Gene, the Drosophila Histone H3 Lysine 79 Methyltransferase

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ABSTRACT

We have identified a novel gene named *grappa* (*gpp*) that is the Drosophila ortholog of the *Saccharomyces cerevisiae* gene *Dot1*, a histone methyltransferase that modifies the lysine (K)79 residue of histone H3. *gpp* is an essential gene identified in a genetic screen for dominant suppressors of pairing-dependent silencing, a *Polycomb-group* (*Pc-G*)-mediated silencing mechanism necessary for the maintenance phase of *Bithorax* complex (*BX-C*) expression. Surprisingly, *gpp* mutants not only exhibit *Pc-G* phenotypes, but also display phenotypes characteristic of *trithorax-group* mutants. Mutations in *gpp* also disrupt telomeric silencing but do not affect centric heterochromatin. These apparent contradictory phenotypes may result from loss of *gpp* activity in mutants at sites of both active and inactive chromatin domains. Unlike the early histone H3 K4 and K9 methylation patterns, the appearance of methylated K79 during embryogenesis coincides with the maintenance phase of *BX-C* expression, suggesting that there is a unique role for this chromatin modification in development.

THE homeotic genes of the *Antennapedia* (*ANT-C*) and *Bithorax* complexes (*BX-C*) are responsible for specifying parasegment identity in the fly. Early in development *gap* and *pair-rule* genes initiate parasegment-specific patterns of *ANT-C* and *BX-C* homeotic gene activity. The expression patterns established during the initiation phase are then sustained during the remainder of development by a maintenance system consisting of the *trithorax-Group* (*trx-G*) and the *Polycomb-Group* (*Pc-G*) genes, which have antagonistic functions (Kennison 1995; Mahmoudi and Verrijzer 2001; Simon and Tamkun 2002). *trx-G* proteins are required to maintain gene activity, and in *trx-G* mutants inactivation of one or more of the *ANT-C* and *BX-C* homeotic genes causes posterior-to-anterior transformations in parasegmental identity. Conversely, *Pc-G* proteins function as silencers, and in *Pc-G* mutants the inappropriate activation of homeotic genes causes anterior-to-posterior transformations in parasegmental identity.

The antagonistic activities and phenotypes associated with *trx-G* and *Pc-G* genes are a function of their distinct effects on chromatin structure. For example, the *trx-G* protein ASH1 is a histone methyltransferase that modifies histone H3 lysine (K) 4 and K9 residues as well as histone H4 K20 (Beisel *et al.* 2002). It is believed that this epigenetic modification functions in turn to recruit another TRX-G protein, Brahma, the fly homolog of the SNF2/SWI2 protein in yeast, which facilitates transcription via chromatin remodeling. Like ASH1, the PC-G protein E(Z) is also a histone methyltransferase, but has a different specificity, methylating K9 and K27 of histone H3 (Czermin *et al.* 2002). Nucleosomes possessing histone H3 methylated at these residues function to recruit Polycomb and other components of the PC-G silencing complex (Czermin *et al.* 2002).

In genetic screens designed to identify novel factors required for *Pc-G*-mediated silencing we recovered several alleles of a new Drosophila gene, *grappa* (*gpp*). *gpp* has the unusual property of exhibiting phenotypes and genetic interactions that are characteristic of both *Pc-G* and *trx-G* genes and is a member of the Enhancer of *trithorax* and *Polycomb* (ETP) class of genes (see Brock and van Lohuizen 2001). Moreover, *gpp* mutants dominantly suppress silencing by telomeric, but not centromeric heterochromatin. We show here that *gpp* is the fly ortholog of the *Saccharomyces cerevisiae Dot1* gene, a gene that was originally identified in an overexpression screen for factors that perturb telomeric silencing (Singer *et al.* 1998). Analysis of *gpp* mutants indicates that, like *Dot1* (Lacoste *et al.* 2002; Ng *et al.* 2002; van Leeuwen *et al.* 2002), GRAPPA functions as a methyltransferase and is required for the methylation of lysine 79 of histone

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TABLE 1

Heteroallelic combinations of *gpp* alleles result in an enhanced *Abd-B* LOF phenotype

	gpp^{1A}	*gpp*^{61A}	*gpp*^{72A}	*gpp*^{94A}	*gpp*^{8}	*gpp*^{IN1}	*gpp*^{X}	*gpp*^{XXV}	*Df(3R) WIN11*
gpp^{1A}	E								
gpp^{61A}	E	L							
gpp^{72A}	E	E	E						
gpp^{94A}	E	E	E	E					
gpp^{8}	E	L	E	E	L				
gpp^{IN1}	E	L	E	E	L	L			
gpp^{IN2}	ND	L	ND	ND	ND	L	L	ND	ND
gpp^{X}	E	L	E	E	L	L	L		
gpp^{XXV}	E	L	E	E	L	L	L	L	
Df(3R) WIN11	E	L	E	E	L	L	L	L	L

E, genetic combination of these *gpp* alleles enhances the *Abd-B* LOF phenotype; L, genetic combination of these *gpp* alleles results in lethality; ND, not done.

H3 (H3meK79). We also describe novel features of the developmental profile and polytene chromosome distribution of the H3meK79 modification.

MATERIALS AND METHODS

***gpp* mutants:** *gpp* mutants were isolated by mating EMS-mutagenized w^1 males containing *Mcp* or *iab-7 PRE mini-white* reporters (lines: *Mcp*, ff#10.5, Muller *et al.* 1999; *iab-7* PRE 18.73.1, Hagstrom *et al.* 1997) to their sibs and screening F_1 progeny for elevated *mini-white* expression. The mutant gene responsible for suppression of PRE-mediated silencing was named *grappa* because of the light yellow phenotype associated with the posterior-to-anterior transformation of the A5 and A6 male tergite plates. (This light color reminded one of the authors of a favorite drink from his not so distant youth and is thus the origin of the name.) X-ray alleles were isolated on the basis of their failure to complement *gpp* EMS mutants. Two of the X-ray alleles, *gpp*^{X} and *gpp*^{XXV}, have breakpoints in 83E4-83E8. Genomic DNA from these alleles was analyzed by probing with sequences amplified from BAC 32D5, which spans *gpp* (Hoskins *et al.* 2000). The primers used for probe 1 were Fwd, GGGCAGCGGCAGCAGATTTGCTGG; Rev, GAT TGTCCGTATAGGAGGGG. Primers for probe 2 were Fwd, GCACTAGCTGAATGCCGCTTTGGC; Rev, CGCTTTAACTT TGAACTAAGTCGACTGC. The *P*-element insertion in *gpp*, *gpp*^{IN1}, was isolated by crossing *pLac w* males to *Δ2-3 transposase* females. Plasmid rescue was used to obtain clones from *gpp*.

Genetic interactions: *gpp* interactions with the various *Pc-G* genes were carried out at 22°. All *Pc-G* lines were obtained from the Bloomington Stock Center. The data from these crosses were obtained by counting the male sex comb teeth present on all legs and averaging this number. The numbers of sex comb teeth present on the first tarsal leg were summed, averaged, and subtracted from the above number to generate the values depicted in Figure 2A. *gpp* alleles were reciprocally crossed against *Abd-B*^{M1}, a null allele of the *Abd-B* gene at 22°. F_1 *trans*-heterozygous males were scored for rotated genitalia. Chi-square analysis was used to determine if the number of rotated genitalia of a particular genotype was statistically different from control crosses.

RNA isolation and Northern blotting: Total RNA was isolated from adult flies using an acid phenol extraction protocol (Samuels *et al.* 1991). Poly(A)$^+$ RNA was isolated from 24-hr-old embryos and third instar larvae using the μMACS mRNA isolation kit (Miltenyi Biotec). RNA (10 μg per lane) was loaded and the blot was probed with *gpp* cDNAs.

Western analysis: Six imaginal discs or central nervous systems (CNSs) were isolated from third instar larvae and immediately homogenized in gel loading buffer (125 mM Tris, pH 6.9, 6% SDS, 50% glycerol, 10% β-mercaptoethanol). The samples were electrophoresed on a 20% SDS PAGE gel and transferred to membranes. A 1:6000 dilution of histone H3 dimethyl K79 (H3dmeK79) antibody was incubated with the blots [a generous gift from Yi Zhang (Ng *et al.* 2002) and Michael Grunstein]. The same results were observed using anti-H3dmeK79 commercially available from Upstate Biotechnology (Lake Placid, NY). Blots were rehybridized with anti-*snf* monoclonal antibody 4G3 serum at a 1:10 dilution as a control. This antibody recognizes the SNF protein involved in nuclear mRNA splicing and is present in all cells.

Immunocytochemistry: Imaginal wing discs isolated from wild-type and *gpp*^{X} third instar larvae were fixed in a 4% solution of formaldehyde in PBS and washed and blocked in PBTX (1× PBS, 0.3% Triton X-100, and 0.3% BSA). Staged embryos were fixed and stained following the protocol of Deshpande *et al.* (1995). Discs and embryos were incubated with K79 antibody (1:6000) and then stained with secondary antibodies (Alexa fluor 546 and 488; Molecular Probes, Eugene, OR).

RESULTS

***gpp* mutants suppress pairing-dependent silencing:** PC-G silencing complexes are targeted to *cis*-acting targets known as *Polycomb* response elements (PREs) that are located in the *ANT-C* and *BX-C* regions. When *mini-white* transgenes containing PREs are paired *in vivo*, a phenomenon known as pairing-sensitive silencing (PSS) represses reporter gene expression. To identify genes involved in PSS we screened for second-site mutations that dominantly suppress silencing induced by the *BX-C* PREs *Mcp* and *iab-7*. While many of the mutations recovered corresponded to known *Pc-G* genes (Vazquez *et al.* 1993), several appeared to represent novel loci. One of the novel genes, called *grappa* (*gpp*), was defined by four independent alleles. Three of the alleles, *gpp*^{1A}, *gpp*^{72A}, and *gpp*^{94A} are viable, while the fourth, *gpp*^{61A}, is lethal (Table 1).

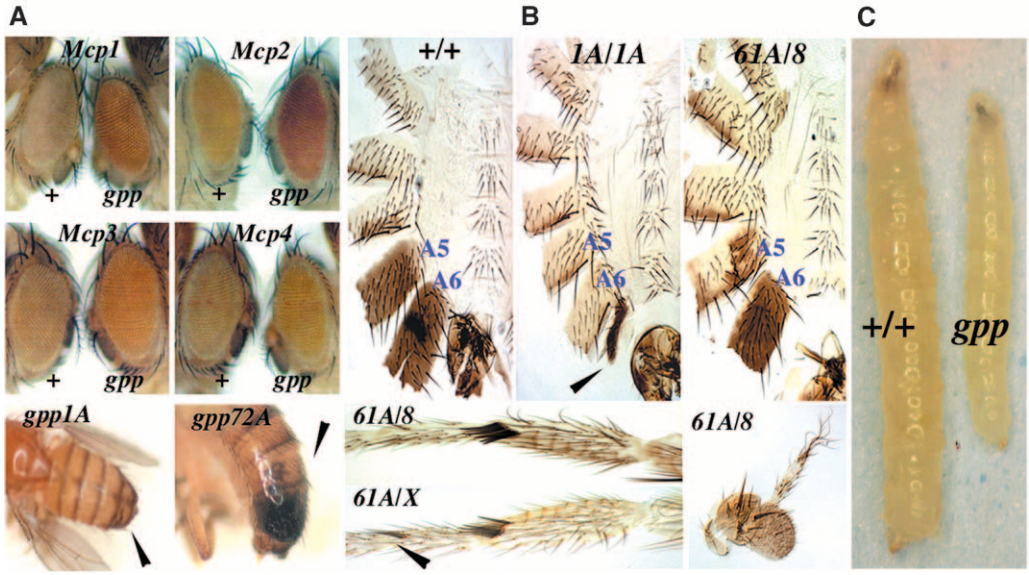


FIGURE 1.—*gpp* mutants suppress *PRE*-mediated chromatin silencing and exhibit numerous phenotypes. (A) gpp^{1A} mutants suppress both *Mcp cis*- and *trans*-silencing. The Mcp1 panel depicts flies containing homozygously paired *Mcp mini-white* reporter transgenes. The fly on the right contains paired *Mcp* transgenes recombined with the gpp^{1A} mutation. In this fly, the gpp^{1A} mutation suppresses *trans*-silencing of the *Mcp* constructs as evidenced by the increase in *mini-white* expression. *Mcp2*, *-3*, and *-4* display fly lines containing *cis-Mcp* constructs (see Table 2). The flies on the right-hand side of each of these contain different *Mcp cis* lines that are *trans*-heterozygous with the gpp^{1A} mutant. In each case, the *cis*-silencing associated with these lines is disrupted by the gpp^{1A} mutation. The bottom displays prominent *trx-G* phenotypes associated with two different *gpp* alleles, gpp^{1A} and gpp^{72A}. The arrowheads identify the posterior-to-anterior transformation of adult male tergite plates associated with these two alleles of *gpp*. (B) A number of additional phenotypes associated with *gpp* alleles. The top depicts tergite filets dissected from wild-type, gpp^{1A}, and gpp^{61A}/gpp^{8} males. Note that in gpp^{1A} there is a complete loss of tergite coloration relative to wild type. This is indicative of the transformation of adult segment A5 and A6 into an A4 anterior segmental identity. Also note that in these flies the ventral sternite hairs are transformed into a more anterior identity. The arrowhead points to a vestigial A7 tergite not normally present in males. In gpp^{61A}/gpp^{8} there is a loss of A5 tergite coloration and additional sternite hairs characteristic of a *trx-G* phenotype. The bottom left shows male legs from gpp^{61A}/gpp^{8} and gpp^{61A}/gpp^{X} combinations that possess a sex comb reduced phenotype. However, the gpp^{61A}/gpp^{X} animals exhibit an extra sex comb on the first leg as indicated by the arrowhead. The bottom right displays an aristae-to-leg transformation associated with gpp^{61A}/gpp^{8} mutant flies. (C) The slow growth phenotype associated with homozygous gpp^{X} larvae.

As illustrated for gpp^{1A} in Figure 1A, all four *gpp* alleles dominantly suppress *Mcp* silencing of *mini-white*. *gpp* mutants also suppress silencing by the *iab-7* and *bxd* PREs (data not shown). On the basis of the effects of these mutations on PRE activity, *gpp* would be classified as a *Pc-G* gene. This classification is supported by the finding that gpp^{61A} pharate adults (as well as two alleles isolated in other screens, gpp^{8} and $gpp^{IN\text{-}1}$) exhibit aristae-to-leg transformations (see Figure 1B; DUNCAN *et al.* 1998). On the other hand, a number of additional phenotypes are observed that suggest that *gpp* is not a typical *Pc-G* gene. First, *gpp* mutants exhibit a rough eye phenotype that differs in severity depending on the allele. Second, as described further below, *trx-G*-like, not *Pc-G*-like transformations are observed in abdominal segments and legs of *gpp* mutants. Third, an additional EMS allele of *gpp*, gpp^{8}, was identified in a screen for suppressors of a dominant gain-of-function *hedgehog* (*hh*) mutation, *Moonrat* (hh^{Mrt}; FELSENFELD and KENNISON 1995). gpp^{8} is embryonic lethal when homozygous and is pharate adult lethal when combined *in trans* with gpp^{61A} (see Table 1). Since hh^{Mrt} activates *hh* transcription in inappropriate tissues and cell types, suppression by a *gpp* mutation also points to a role in transcriptional activation rather than silencing. Given this unusual combination of phenotypes, it was of interest to further characterize *gpp*.

Isolation of *gpp* rearrangements: Recombination mapping relative to *P*-element insertions along the third chromosome placed *gpp* in the centromere proximal region of 3R. It was localized to the 83-84 interval by the failure of gpp^{61A} (and several other lethal alleles) to complement *Df(3R) WIN11* and *Df (3R)Dfd13*. To further analyze the genetic properties of *gpp* and obtain DNA rearrangements that would facilitate molecular isolation, *P*-element- and X-ray-induced *gpp* alleles were isolated. The *P*-element allele, $gpp^{IN\text{-}1}$, is homozygous lethal at the pharate adult stage. It is also lethal in combination with *Df(3R)WIN11*, gpp^{61A}, or gpp^{8}. *In situ* hybridization localized the *P*-element insertion to 83E on 3R. The gpp^{X} X-ray allele exhibits no obvious patterning defects and is lethal during late larval stages. The growth rate of gpp^{X} larvae is reduced relative to similarly aged wild-type larvae (Figure 1C). Cytological examination of gpp^{X} reveals a small inversion with breakpoints in 83C8-D1, 2 and 83 E4-8. The other X-ray allele, gpp^{XXV}, is embryonic lethal, and it has both an inversion (75 C1, 2; 83 E4-8) and a small deletion (of 75C1, 2-E). The small deletion rather than the lesion in *gpp* seems to be responsible for the embryonic lethality of gpp^{XXV}.

Phenotypic effects of *gpp* mutants: *gpp suppresses Pc-G-mediated silencing:* To compare the effects of different *gpp* alleles on PSS of *mini-white* by PREs, we took advantage of the unusual ability of *Mcp* to establish silencing

TABLE 2

Effect of *gpp* mutations on *Mcp*-mediated long-range silencing

	gpp alleles						
Mcp cis-silencing lines[a]	gpp^{1A}	gpp^{72A}	gpp^{94A}	gpp^{61A}	gpp^{8}	gpp^{X}	gpp^{XXV}
Mcp2 (ff#13.101 w#14.29)	S	S	ws	ws	S	N	ws
Mcp3 (w11.16, 11.102)	S	ws	ws	N	ws	S	ws
Mcp4 (wy2.63, ff#15.30)	S	ws	N	N	N	N	N

The tabulated results represent *trans*-heterozygous combinations of *Mcp* lines with different *gpp* alleles. S, suppression of silencing; N, no effect; ws, weak suppression of silencing.

[a] See Muller *et al.* 1999.

interactions *in cis* between transgenes inserted at distant sites on the same chromosome (Muller *et al.* 1999). We tested all of the *gpp* alleles (except for the *P* transposon induced) with three different *Mcp-mini-white* transgene *cis* combinations. Most of the alleles suppress long distance *Mcp* silencing; however, the strength of suppression varies with the *gpp* allele and the *Mcp cis* combination (Table 2). gpp^{1A} shows the most dramatic effects on *Mcp* silencing, and it strongly suppresses all three *Mcp cis* combinations. gpp^{72A} also suppress the three *cis* combinations; however, the increase in *mini-white* expression is not as pronounced for two of the *cis* combinations as it is with gpp^{1A}. While none of the remaining EMS and X-ray alleles suppress the *Mcp4* combination, all suppress one or both of the other combinations. For example, gpp^{8} and gpp^{XXV} suppress *Mcp* silencing in both the *Mcp2* and the *Mcp3* combinations, while gpp^{X} suppresses silencing only in the *Mcp3* combination.

In addition to testing the *cis Mcp* lines, we examined the effect of the gpp^{1A} allele on various hemizygous and homozygous PRE-containing transgene lines. We found that gpp^{1A} increases the eye color of both hemizygous and homozygous *Mcp*, *iab-7* PRE and *bxd* PRE lines but has no effect on the endogenous *white* locus (data not shown).

gpp interacts with Pc-G genes: Males heterozygous for *Pc-G* mutations often exhibit ectopic sex comb teeth or complete sex combs on the second and third tarsal legs. These transformations result from a loss of *Pc-G* silencing and can be enhanced by double-mutant combinations with other *Pc-G* genes. To explore the role of *gpp* in *Pc-G*-dependent silencing, we tested whether *gpp* dominantly enhances the phenotypic effects of mutations in *Sex combs extra* (*Sce*), *Polycomblike* (*Pcl*), Sex combs on the midleg (*Scm*), and *Polycomb* (*Pc*).

The simplest interactions are observed for gpp^{8} and the two X-ray alleles, gpp^{X} and gpp^{XXV}. As shown in Figure 2 these alleles increase the number of sex comb teeth in males heterozygous for all four *Pc-G* mutations. Moreover, for all three alleles, the strongest interactions are observed for *Scm* and Pc^{3}. As the two X-ray alleles are presumed to be simple loss-of-function, rather than antimorphic or neomorphic mutations, these findings would be consistent with the classification of *gpp* as a *Pc-G* gene. More complex interactions are observed for gpp^{1A} and gpp^{94A}. While gpp^{1A} enhances the sex combs phenotype of *Sce*, *Pcl*, and *Scm*, it has the opposite effect on Pc^{3}, suppressing the sex combs phenotype. Similarly, gpp^{94A} acts as both an enhancer and a suppressor of the sex comb phenotype, depending upon the particular *Pc-G* mutation. Why these two *gpp* alleles do not interact in a consistent pattern with the *Pc-G* mutations is unclear; it is possible that this reflects some unusual properties of the mutant GRAPPA (GPP) protein.

gpp affects telomeric silencing but not centromeric silencing: The suppression of *Pc-G*-mediated silencing of *mini-white* as well as the genetic interactions with mutations in several *Pc-G* genes would be consistent with the idea that *gpp* is a member of the *Pc-G* family. While the silencing activity of many *Pc-G* family members is restricted to euchromatic genes, a subset of the PC-G proteins function in telomeric silencing [telomeric position effect (TPE); Cryderman *et al.* 1999; Boivin *et al.* 2003]. To determine whether *gpp* is required for TPE, we asked whether *gpp* mutations dominantly suppress the silencing of *mini-white* transgenes inserted into telomeric heterochromatin of the second, third, and fourth chromosomes (Cryderman *et al.* 1999; Figure 3A). gpp^{XXV}, gpp^{X}, and gpp^{61A} mutants suppressed TPE of all *mini-white* transgene inserts. As illustrated for gpp^{X} in Figure 3A, suppression by these three *gpp* alleles is quite strong relative to eye color controls. The one exception is the telomeric insert in 2L, which is suppressed only weakly by gpp^{X} and the two other *gpp* alleles; however, this insertion is also only weakly suppressed by mutations in other *Pc-G* genes required for TPE, such as $Su(z)2^{5}$ (our unpublished data; Cryderman *et al.* 1999). Three other alleles, gpp^{8}, gpp^{72A} and gpp^{94A}, also suppressed TPE at most but not all telomeric sites.

We also tested whether *gpp* has any dominant effects on silencing by centromeric heterochromatin [position effect variegation (PEV)]. None of the *gpp* alleles had any effect on PEV silencing associated with *mini-white* insertions into second or third chromosome centromeric heterochromatin (Figure 3B). Assuming that the unusual effect of gpp^{8} on the fourth chromosome insert

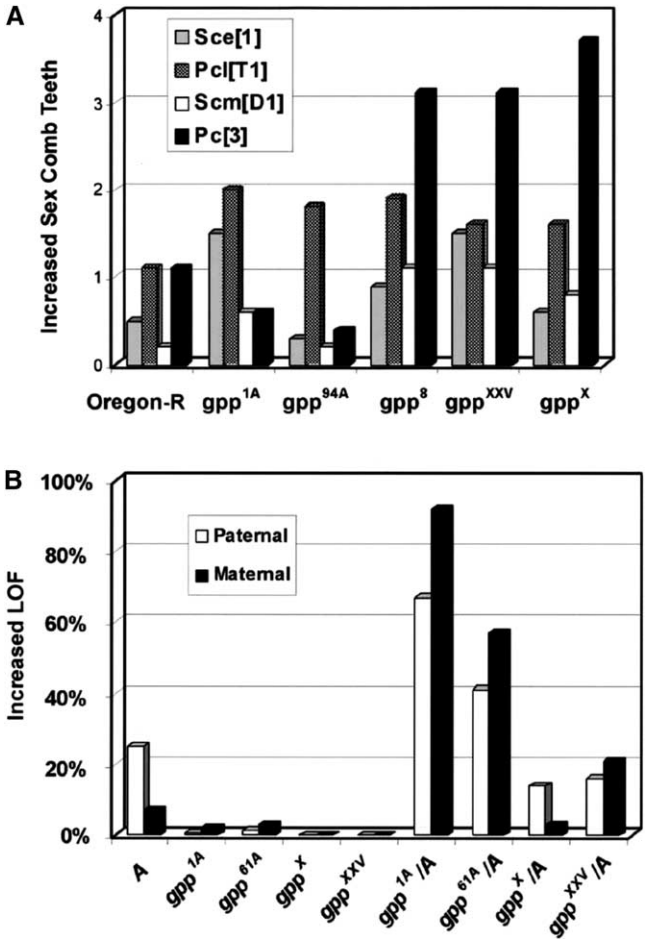


FIGURE 2.—*gpp* alleles genetically enhance *Pc-G* and *Abd-B* mutant phenotypes. (A) *gpp* alleles were crossed against different *Pc-G* mutants. The *y*-axis shows the relative increase in the sex comb teeth associated with the double mutants. The number of sex comb teeth was calculated by counting the total number of teeth on all legs, averaging this number, and subtracting the averaged number of teeth present on the first tarsal leg. A minimum number of 54 flies were counted for each genotype. *Sce*^{1}*/TM6B, Sex combs extra*; *Pcl*^{T1}*/Cyo, Polycomb-like*; *Scm*^{D1}*/TM6,Sb, Sex combs on the midleg*; *Pc*^{3}*/TM3,Ser, Polycomb.* (B) *gpp* alleles reciprocally crossed against w^1;+;*Abd-B*^{M1}*/TM6B* mutants enhance the male rotated genitalia phenotype. The increase in rotated genitalia of the *trans*-heterozygous combinations of *gpp* and *Abd-B*^{M1} is statistically significant. All *P*-values were $<10^{-4}$ by the χ^2 test. *y*-Axis, percentage change in genitalia phenotype; *x*-axis, *trans*-heterozygous combinations of *gpp* alleles and either w^1 or w^1;+;*Abd-B*^{M1}*/TM6,B,TB* flies; LOF, loss of function; A, the w^1;+;*Abd-B*^{M1} allele.

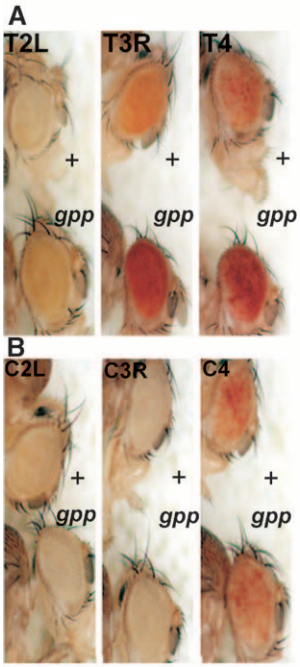
is unique to this particular allele, these findings suggest that *gpp* does not function in centromeric silencing.

gpp is also a member of the trx-G gene family: While the effects of *gpp* mutations on *Pc-G* and telomeric silencing suggest that it functions in the establishment and/or maintenance of repressive chromatin structures, *gpp* mutants also exhibit phenotypes and genetic interactions that are characteristic of mutations in *trx-G* genes. *trx-G*-like phenotypes were first observed in *gpp*^{61A}/+ males as a transformation of the A5 tergite into a tergite that resembles A4. This phenotype is characteristic of mutations that compromise *Abd-B* activity in parasegment 10 and would be expected for a mutation in a *trx-G*, not a *Pc-G* gene. While *gpp*^{61A}*/gpp*^{61A} flies don't survive to the adult stage, males homozygous for *gpp*^{1A}, *gpp*^{72A}, and *gpp*^{94A} can be obtained. In these males A5 and also A6 is transformed toward an A4 identity. The severity of this *Abd-B*-like loss-of-function phenotype ranges from the moderate transformation seen in *gpp*^{72A} and *gpp*^{94A} to near complete loss of male pigmentation in both A5 and A6 and the appearance of an "A7" tergite in homozygous *gpp*^{1A} (see Figure 1, A and B). Also indicative of A6 to A5/A4 transformation, *gpp*^{1A}, *gpp*^{72A}, and *gpp*^{94A} males have hairs on the sixth sternite. Similar transformations of A5 and A6 toward A4 are observed when viable *gpp* alleles are combined with some of the lethal alleles (see *gpp*^{1A}*/gpp*^{8} in Figure 1B). The severity of the transformations in segment identity seen in homozygous mutant males is influenced by the maternal genotype. For example, the phenotype in the progeny is more severe when the mothers are homozygous for the *gpp* mutation than when the mothers are heterozygous.

While the *Abd-B* loss-of-function (LOF) phenotype is not observed in flies heterozygous for *gpp*^{8}, for either X-ray allele or the *P*-element insertion *gpp*$^{IN-1}$, dissection of homozygous male *gpp*$^{IN-1}$ pupae reveals that A5 and A6 are transformed toward an A4 identity. The *gpp*$^{IN-1}$ males also have a reduced number of sex combs, indicating that expression of homeotic genes in the *ANT-C* is altered. Similar loss-of-function transformations of A5 and A6 and a reduction in the number of sex combs are observed in dissected *gpp*^{61A}*/gpp*^{61A} pupae (not shown). A reduction in the number of sex comb teeth (and sex combs on the fourth tarsus) is also observed in dissected *gpp*^{61A}*/gpp*^{8} and *gpp*^{61A}*/gpp*^{X} pupae (see Figure 1B). In addition, as shown in Table 1, *gpp*^{X}, *gpp*^{XXV}, *gpp*$^{IN-1}$, and *gpp*^{8} all enhance the transformation of A5 and A6 when *trans* to a viable *gpp* allele.

Since these phenotypes suggest that *gpp* functions in promoting gene expression, we tested whether *gpp* mutations dominantly enhance the weak haplo-insufficiency of the *Abd-B* gene (Figure 2B). While neither X-ray allele shows strong interactions, both *gpp*^{1A} and *gpp*^{61A} are potent enhancers of the *Abd-B* haplo-insufficiency. Moreover, this interaction is consistently stronger when the *gpp* mutation is inherited from the mother. These findings, taken together with the *trx-G*-like phenotypes seen in different *gpp* mutant backgrounds would be consistent with the idea that *gpp* has some type of function in gene activation or the maintenance of gene activity.

Molecular isolation of the *gpp* gene: Clones containing DNA flanking the *gpp*$^{IN-1}$ *P*-element were isolated by plasmid rescue and the insertion site was determined by comparison with the genomic sequence. On the centromere proximal side, the transposon is 1.5 kb from the 5′ end of the Celera predicted gene *CG1021*, while



Telomere Lines	gpp^{XXV}	gpp^{X}	gpp^{8}	gpp^{61A}	gpp^{72A}	gpp^{94A}	gpp^{1A}
39C-50 (2R)	S	S	S	S	ws	ws	N
39C-27 (2R)	S	*nd*	*nd*	S	N	N	N
39C-5 (2L)	ws	ws	ws	ws	N	N	N
39C-31 (3R)	S	*nd*	ws	S	S	S	N
39C-55 (3R)	S	S	*nd*	S	S	S	N
39C-62 (3R)	S	*nd*	ws	S	S	S	N
36C-72 (4)	S	S	E	S	S	ws	N
118E-15 (4)	S	S	N	S	S	ws	N

Centromere Lines	gpp^{XXV}	gpp^{8}	gpp^{61A}	gpp^{72A}	gpp^{94A}	gpp^{1A}
39C-4 (C2L)	N	N	N	N	N	N
118E-12 (C3R)	N	N	N	N	N	N
118E-10 (C4)	N	ws	N	N	N	N

S = Suppression
ws = weak suppression
E = Enhancement
nd = not done

FIGURE 3.—*gpp* mutants suppress TPE but not PEV. (A) Flies heterozygous for *P*-element reporters inserted into the subtelomeric chromatin of chromosomes 2L, 3R, and 4. The three bottom flies are *trans*-heterozygous combinations of the telomere inserts and the gpp^{X} allele. TPE is suppressed in these flies as evidenced by the increase in *mini-white* expression of the telomeric inserts relative to the heterozygous controls. The telomere lines $y^{1}w^{1}$;39C-5;+;+, $y^{1}w^{1}$; +;39C-55;+, and $y^{1}w^{1}$;+;+;118E-15 were used (CRYDERMAN *et al.* 1999). The table to the right documents the effect on TPE of *trans*-heterozygous combinations of various *gpp* alleles with different second, third, and fourth chromosome telomere *P*-element inserts. The genetic effect on suppression or enhancement of TPE by *gpp* mutations was measured by comparing *trans*-heterozygous eye colors relative to heterozygous controls. S, strong suppression of TPE; ws, weak suppression of TPE; N, no effect on TPE; E, enhancement of TPE; nd, not done. (B) Flies heterozygous for centromeric reporter *P* elements. Bottom flies are *trans*-heterozygous combinations of the different centromeric inserts and the gpp^{X} allele. PEV silencing is not affected by *gpp* mutations. The centromeric *P*-element lines $y^{1}w^{1}$;39C-4;+, $y^{1}w^{1}$;+;118E-12, and $y^{1}w^{1}$;+;+;118E-10 were used in this figure (from CRYDERMAN *et al.* 1998). The table to the right documents the effect of various *gpp* alleles on the centromeric *P*-element inserts. The genetic effect on suppression or enhancement of PEV by *gpp* mutations was measured as above against controls. N, no effect on PEV; ws, weak suppression of PEV.

on the centromere distal side it is ~30 kb from the predicted 5′ end of *CG10272*. An additional *P*-element line, $gpp^{IN\text{-}2}$ [$l(3)03342^{03342}$; SPRADLING *et al.* 1999] is inserted into the first intron of *CG10272* and is ~17 kb distal to the $gpp^{IN\text{-}1}$ *P*-element insertion site (see Figure 4A). The sequence insertion site for the $gpp^{IN\text{-}2}$ *P*-element was determined by the Berkeley gene disruption project (SPRADLING *et al.* 1999). The $gpp^{IN\text{-}2}$ *P*-element insertion does not complement lethal *gpp* alleles (Table 1). In addition to the *P*-element insertions, the position of the X-ray breakpoint alleles was determined. We found that the gpp^{X} breakpoint maps to a restriction fragment located ~13 kb distal to the $gpp^{IN\text{-}1}$ *P*-element and 4 kb proximal to $gpp^{IN\text{-}2}$ (Figure 4A). However, the gpp^{XXV} breakpoint is located ~35 kb from $gpp^{IN\text{-}1}$ inside the predicted coding region for the *CG10272* gene. This finding suggested that *CG10272* might correspond to *gpp*.

Structure of the *gpp* transcription unit: We further characterized the *gpp* transcription unit by analyzing cDNAs isolated from various ovarian, embryonic, and larval libraries, by RT-PCR and by Northern blotting. As shown in Figure 4A, *gpp* is 42 kb in length and encodes mRNAs that range in size from 6.5 to 10 kb. The 5′ end of the gene is just upstream of the $gpp^{IN\text{-}1}$ transposon, and because of alternative splicing several different 5′-UTRs can be produced. The alternative exon sequences at the 5′ end of the transcription unit are separated from the main body of the gene by an ~30-kb intron and they are spliced to a common exon containing additional 5′-UTR sequences plus the translation start site for all known *gpp* mRNAs. The main body of the mRNA has 5 exons that are common to all *gpp* mRNAs and encode the bulk of the protein. There are multiple 3′ exon combinations that differ from each other by the use of alternative 3′ and 5′ splice sites and poly(A) addition sites. *gpp* could potentially code for 12 different mRNAs; however, only six variants were detected in our analysis.

In 0-24 embryos the major species is ~9 kb and there are less abundant RNAs of 7.5 and 10 kb (Figure 4B). This profile changes as development proceeds. In larvae (not shown), the 9- and 7.5-kb species are equally abundant while a smaller 6.5-kb species appears. In adults (see Figure 4B) the predominant mRNA is 7.5 kb. On the basis of our analysis of the different mRNAs, the 9-kb species seen in embryos and larvae is likely to correspond to T2, while the 7.5-kb species seen in embryos, larvae, and adults could correspond to T1, T4, and T5 as all three of these mRNAs are between 7.3 and 7.7 kb. T3 is 10 kb, about the size of the very large mRNA in embryos, while T6 is 6.6 kb and could correspond to the small mRNA seen at the larval stages.

Inspection of the *gpp* transcription unit reveals that the $gpp^{IN\text{-}1}$ *P*-element is inserted in the first exon, the

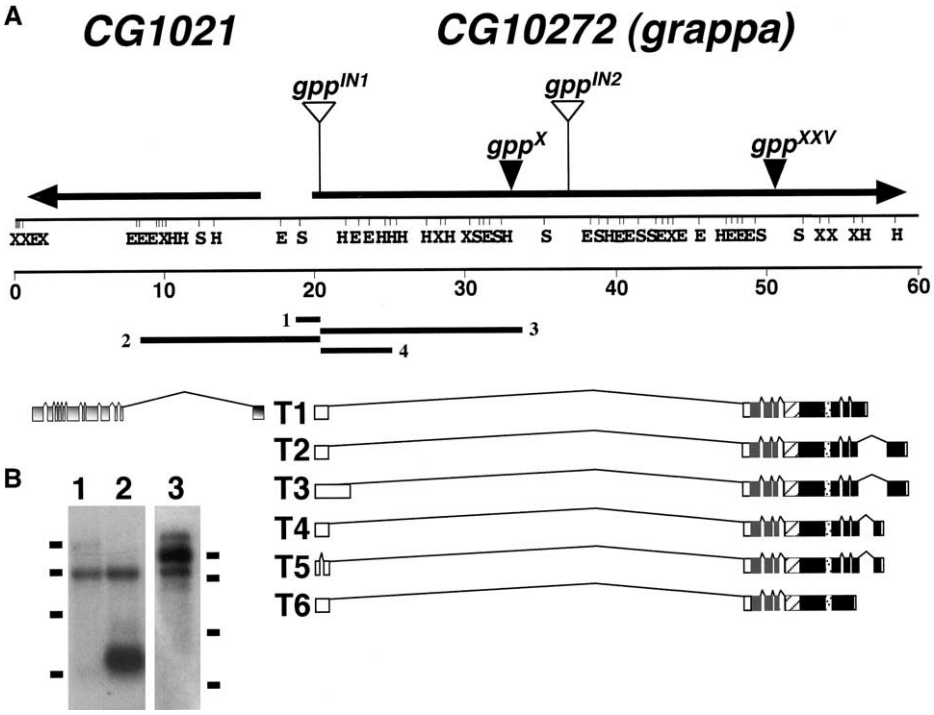


FIGURE 4.—The molecular organization of the *gpp* locus and the developmental expression of *gpp* RNAs. (A) Genomic organization and restriction map of the *gpp* locus along the 83 E4-8 region on the third chromosome. The location and the direction of transcription (solid arrows) of the *CG1021* and *CG10272* (*gpp*) genes are depicted at the top. The location of the *P*-element alleles gpp^{IN-1} and gpp^{IN-2}[*l(3)03342*; see SPRADLING *et al.* 1999] and the X-ray breakpoint alleles (gpp^{X} and gpp^{XXV}) are depicted above the *gpp* transcription unit. A restriction enzyme map and a scale bar (from 0 to 60 kb) are presented below the transcription units. Plasmid rescue clones (1–4) isolated from gpp^{IN-1} flies are positioned around the proximal *P*-element insert. A putative *CG1021* transcript is pictured on the left side of the figure (in gray). Six alternatively spliced *gpp* cDNAs are shown below the map. The white boxes on the cDNAs represent 5′- and 3′-UTRs. Solid boxes represent gene exons. Transcripts T1–T6 represent different alternatively spliced *gpp* transcripts. The size (in kilobases) of the transcripts is as follows: T1, 7.6; T2, 8.7; T3, 10.1; T4, 7.6; T5, 7.3; and T6, 6.6. Protein domains overlaying respective encoding exons are depicted on the cDNAs: gray box, the domain homologous to DOT1; hatched box, a potential coiled-coil domain; stippled box, a potential ATP/GTP binding domain. Restriction enzyme sites: S, *Sal*I; H, *Hin*dIII; E, *Eco*RI; X, *Xho*I. (B) Northern analysis of *gpp* transcripts. Lanes 1 and 2 contain total RNA isolated from adult w^{1}; *TM3,Ser/Sb*^{1} and gpp^{XXV}/*TM3, Ser* flies, respectively. Lane 3 contains mRNA isolated from 24-hr-old w^{1} embryos. Size markers (in kilobases) are represented as bars on either side of the blots, representing molecular sizes of 9.5, 7.46, 4.4, and 2.37 kb.

gpp^{IN-2} *P*-element is inserted midway through the first intron, the gpp^{X} breakpoint is located in the large intron, and the gpp^{XXV} breakpoint is in the protein coding sequence. Since these mutations disrupt the *gpp* gene, they might be expected to produce aberrant transcripts. To determine if this is the case, Northern blots of RNA from heterozygous flies were probed with *gpp* cDNAs. While we did not detect any unusual transcripts for gpp^{IN-1}/*TM3* or gpp^{X}/*TM6*, an ~4-kb RNA species is present in RNA prepared from gpp^{XXV}/*TM3* flies (Figure 3B). Moreover, consistent with the disruption of the *gpp* transcription unit in gpp^{XXV}, this species is detected with probes upstream of the breakpoint, but not downstream.

The predicted GPP protein: The *gpp* mRNAs code for proteins with a predicted mass ranging from 171 to 232 kD. Homology searches revealed that the common N-terminal domain of GPP shares significant sequence similarity to the *S. cerevisiae* DOT1 protein. DOT1 is a novel histone H3 methyltransferase that modifies the K79 residue inside the first globular domain of H3 (LACOSTE *et al.* 2002; NG *et al.* 2002; VAN LEEUWEN *et al.* 2002). The sequence conservation between the GPP N terminus and DOT1 is ~42%. However, the GPP sequence shows 100% homology to the DOT1 MT methyltransferase fold, which is required for methylation of histone H3 (FENG *et al.* 2002). This suggests that GPP is likely to be a Drosophila H3 K79 methyltransferase. GPP has additional protein domains that are not present in DOT1. These include a putative coiled-coil domain and an ATP/GTP binding domain. The presence of these domains suggests that GPP may dimerize or interact with other proteins. The GPP protein also contains several regions rich in alanine, proline, histidine, and glutamine. While yeast DOT1 does not contain these additional domains, the coiled-coil motif and the proline-rich region are found in human, *Caenorhabditis elegans*, *Drosophila pseudoobscura*, and *Anopheles gambia* DOT1-like proteins.

***gpp* functions as a histone H3 K79 methyltransferase:** The sequence similarity between *gpp* and *Dot1* suggested that GPP protein might function as an H3 K79 methyltransferase. If this is correct, one might expect to find that H3meK79 is depleted in *gpp* mutants. Since our genetic experiments indicated that the *gpp* gene product is supplied maternally and the phenotypic effects of *gpp* mutants in the zygote are not observed until the larval stage, we examined K79 methylation in larval tissues. Imaginal discs from wild-type and gpp^{X} mutant third instar larvae were probed with antibodies raised against H3dmeK79 (NG *et al.* 2002). Figure 5A shows that H3dmeK79 can be detected in all nuclei of wild-type wing discs. In contrast, the level of H3dmeK79 antibody staining is substantially reduced in wing discs from homozygous gpp^{X} larvae. In the second experiment, Western

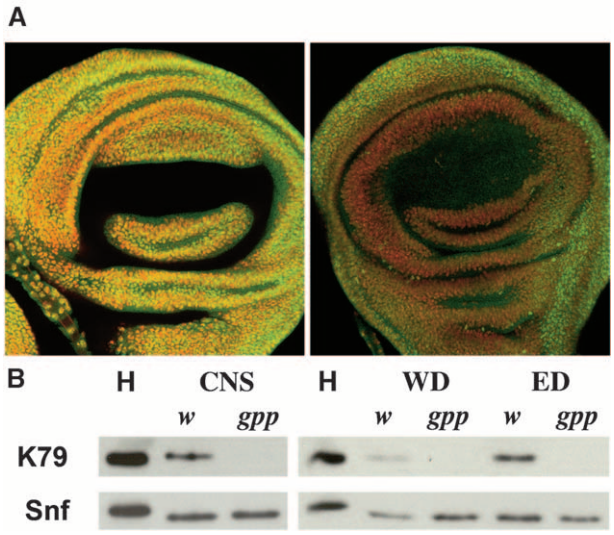


FIGURE 5.—*gpp*^{X} mutant flies are deficient in methylation of H3dmeK79 relative to wild type. (A) Fluorescent images of imaginal wing discs from w^1 (left) and *gpp*^{X}/*gpp*^{X} (right) third instar larvae stained with anti-H3dmeK79 antibody identified with fluorescent secondary antibody (green) and DNA dye (red). Both wing discs were imaged at the same fluorescent intensity using confocal microscopy. (B) Western blot analysis of different imaginal tissue removed from w^1 and *gpp*^{X} third instar larvae. K79 levels are absent in the *gpp*^{X} lanes relative to wild type. H, acid-extracted histone proteins used as a control; central nervous systems (CNS), wing discs (WD), and eye discs (ED) were removed from third instar larvae and prepared as described in MATERIALS AND METHODS. Snf antibody was used to verify equal loading of protein in the lanes.

blots of proteins from wing discs, eye discs, and the CNS of wild-type and *gpp*^{X} mutant larvae were probed with antibodies against either monomethyl (not shown) or dimethyl K79 (Figure 5B). While H3 mono- and dimethyl K79 could be detected in proteins extracted from wild-type larval tissue, we found little or no mono- or dimethyl K79 in tissues from *gpp*^{X} mutants. This would suggest that like its counterparts in other organisms, GPP functions as a histone H3 K79 methyltransferase.

Developmental regulation of H3 K79 methylation: We have previously examined the pattern of histone H3 methylated on K4 and K9 during embryogenesis (SCHANER *et al.* 2003). The developmental profile of these two H3 modifications is consistent with findings in other organisms indicating that histone H3 methylated K4 (H3meK4) is generally associated with active chromatin, while histone H3 methylated K9 (H3meK9) is usually a marker for inactive or silenced chromatin (SCHANER *et al.* 2003). During the rapid nuclear cleavage stages there is little if any H3meK4, while H3meK9 can be detected. H3meK4 remains low even after the nuclei migrate to the periphery of the embryo at nuclear cycle 9–10 and only around cycle 12 does the level of H3meK4 begin to increase in somatic cells. Concomitant with the increase in H3meK4, transcription is upregulated in somatic nuclei at about this stage. At cellularization all somatic nuclei have high levels of H3meK4. In contrast, there is little or no K4 methylation in the pole cells, while there are high levels of K9. This difference reflects the fact that germ cells are transcriptionally quiescent until much later in development. The correlation between transcriptional activity and methylation of K4 or K9 suggested that it would be of interest to examine the developmental profile of K79 methylation.

Like K4, little if any H3 mono- or dimethyl K79 could be detected in early nuclear cleavage stage embryos. However unlike K4, K79 methylation does not appear to be activated when transcription commences in syncytial blastoderm embryos. As shown for a precellular blastoderm embryo in Figure 6, H3meK79 seems to be absent not only from the somatic nuclei, which are still dividing in these embryos, but also from the pole cell nuclei, which are arrested in the cell cycle in G_2 (SU *et al.* 1998). H3 mono- (not shown) and dimethyl K79 (Figure 6) is first readily detected by antibody staining much later in development in germband extended embryos. At this stage H3meK79 accumulation appears to be coupled to the cell cycle. The highest levels of K79 methylation are found in cells undergoing mitosis and as shown by the stage 8/9 embryo in Figure 6 these cells are often clustered in small mitotic domains. In addition to the cells that are in mitosis, some nuclei have high levels of dimethyl K79 histone H3 but do not appear to be in the process of dividing. Usually in these nuclei, antibody staining is concentrated in one or two spots, while the distribution of chromosomal DNA appears to be more diffuse. The remaining nuclei in the germband extended embryos have either low levels of antibody staining or apparently none at all. This general pattern persists through stages 10–11 until just before germband retraction. At this point the level of mono- (not shown) and dimethyl K79 histone H3 (Figure 6) begins to increase substantially, particularly in epidermal nuclei. As illustrated by the stage 15–16 embryo, high levels of dmeK79 histone H3 are observed in virtually all epidermal nuclei at this stage and in subsequent stages. A different pattern is seen in the CNS: in some CNS nuclei there seems to be a high level of dmeK79, while in many other nuclei there is only a low level of this modification. The CNS differs from epidermis at this stage of development in that the neuroblasts and ganglion mother cells (GMCs) in the CNS are still dividing, while only the progeny of the GMCs, the neurons, have stopped dividing (LEE and ORR-WEAVER 2003).

On the basis of our genetic analysis of *gpp* mutants, we anticipated that there would be a substantial maternal contribution of *gpp* gene products and that dmeK79 would be detected in *gpp* homozygous mutant embryos. This expectation was correct since the level of dmeK79 in both *gpp*^{X} mutants and wild type is similar in pattern through the blastoderm to stage 11–12 embryos. However, in older embryos the level of H3dmeK79 appears to be reduced compare to wild-type embryos at a similar

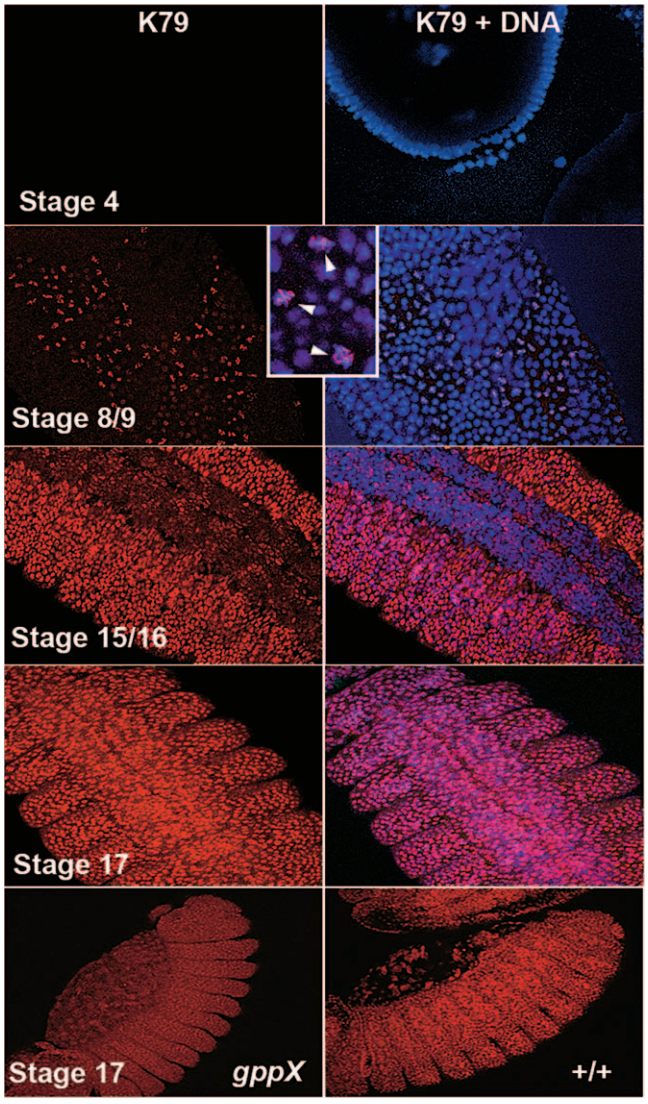


FIGURE 6.—Methylation of H3 K79 in embryos is coincident with the developmental stage of homeotic gene expression. w^1 embryos from different developmental stages were fixed and stained with anti-H3dmeK79. Inset shows a close-up view of mitotic cells stained with K79. Note the difference in the stage 17 embryo CNS K79 staining *vs.* stage 15/16. Bottom compares K79 staining intensity in late-stage gpp^X homozygotes and w^1 embryos. H3 K79 is in red and DNA is blue.

stage. The difference in the level of antibody staining between wild-type and gpp^X embryos in two stage 17 embryos is shown Figure 6. At this stage, the level of H3dmeK79 in the mutant is about one-half that in wild type when imaged at the same intensity.

Distribution of H3dmeK79 in polytene chromosomes: The phenotypic effects of *gpp* mutants point to a role in both gene activation and silencing. Consequently, we examined the distribution of H3dmeK79 in salivary polytene chromosomes to determine if this modification was preferentially localized to active or inactive chromatin. As depicted in Figure 7 several findings are of interest. First, H3dmeK79 appears to be underrepresented at the telomeres compared to other nearby chromosomal segments (Figure 7, A and B). Second, H3dmeK79 is often enriched in puffs and interbands (Figure 7, C, D, and E). Since puffs and interbands are thought to correspond to active chromatin domains, this suggests that there may be a connection between methylation of histone H3 K79 and transcription. However, this connection must be domain specific in that H3dmeK79 is not enriched at all puffs and interbands. Third, H3dmeK79 can also be found localized in bands. As observed for puffs and interbands, H3dmeK79 enrichment in bands is domain specific. Fourth, as evident from the green-to-yellow to orange-to-red staining in different bands, interbands, and puffs, the relative level of H3dmeK79 per "unit" of DNA seems to vary substantially from one chromosomal domain to the next.

DISCUSSION

Recent studies on telomeric silencing in *S. cerevisiae* have led to the identification of a histone methlylase, DOT1, which has a number of unusual properties (SINGER *et al.* 1998; FENG *et al.* 2002; LACOSTE *et al.* 2002; VAN LEEUWEN *et al.* 2002). First, unlike the previously identified histone methylases, DOT1 does not have a canonical SET domain. Instead, the DOT1 protein resembles a family of *S*-adenosyl methione methyltransferases that modify arginine residues (FENG *et al.* 2002; LACOSTE *et al.* 2002; VAN LEEUWEN *et al.* 2002). DOT1 methylates histone H3 at lysine 79 only when it is assembled into nucleosomes and methylation strongly depends upon prior Rad6 dependent ubiquitination of histone H2B at K123 (BRIGGS *et al.* 2002). Second, in yeast, deletion or overexpression of *Dot1* disrupts TPE and also silencing of the mating-type loci (SINGER *et al.* 1998). In contrast, silencing in the yeast ribosomal gene cluster is disrupted only when DOT1 is overexpressed (SINGER *et al.* 1998). Third, both telomeric and mating-type silencing are disrupted by mutations in the lysine 79 residue of histone H3. Fourth, methylation of K79 appears to influence the recruitment of the SIR silencing proteins to the telomeres (VAN LEEUWEN *et al.* 2002; NG *et al.* 2003a). The SIR silencing proteins appear to preferentially associate with chromatin that is deficient in K79 methylation, while the proteins are generally not associated with chromatin in which there is an enrichment for K79 methylated H3 (VAN LEEUWEN *et al.* 2002; NG *et al.* 2003a). Fifth, there is evidence that K79 methylation is coordinated with polymerase transcription via the COMPASS complex (KROGAN *et al.* 2003). Consistent with the idea that K79 methylation might be coordinated with transcription, H3meK79 is enriched in transcribed sequences in yeast and mammals (IM *et al.* 2003; NG *et al.* 2003b). Interestingly, the distribution of H3meK79 in the β-*globin* locus differs from H3meK4 in that it is not found at the locus control region (IM *et al.* 2003). These findings have led to a model in which H3meK79 serves as a marker for

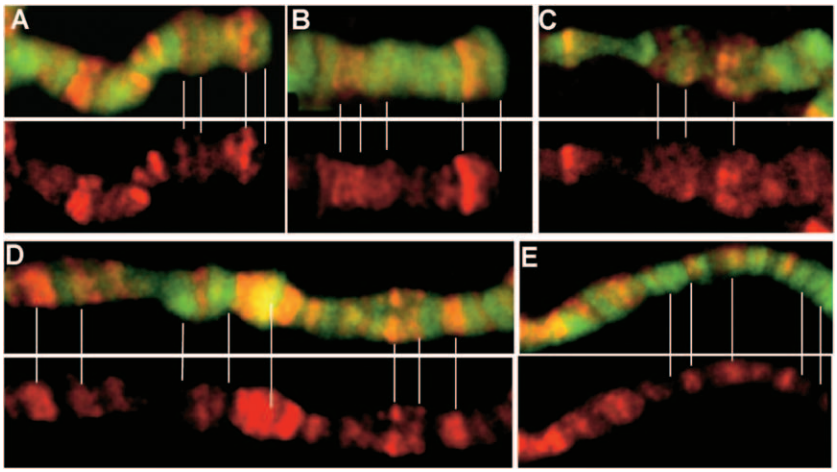


FIGURE 7.—Anti-H3dmeK79 staining of w^1 polytene chromosomes reveals that dmeK79 is found in both band and interband regions, but is not present at the telomeres. (A–D) Top, merged image of the H3dmeK79 (red) and DNA (green) stained chromosomes; bottom, polytenes stained only with K79. White lines connecting the bottom and top identify regions of interest. (A and B) K79 staining is missing from telomeres. (C) K79 staining is present in a puff. (D and E) The distribution of K79 is diverse in its localization to band and interband regions.

transcribed sequences where it functions to block the association of chromatin proteins that mediate transcriptional silencing.

While *Dot1* homologs have been identified in higher eukaryotes, little is known about their biological functions (FENG *et al.* 2002). In this report we have characterized the Drosophila *Dot1* ortholog *gpp*. The *gpp* transcription unit is >40 kb in length and it encodes a complex array of alternatively spliced transcripts that range in size from 6.5 to >9 kb and are expressed at different developmental stages. Consistent with our assignment of the *gpp* gene, *P*-element and X-ray mutations disrupt this large transcription unit and in at least one case lead to the production of truncated mRNAs. The *gpp* transcripts are predicted to encode 170- to 232-kD polypeptides that share a common N-terminal domain that corresponds to about two-thirds of the protein but have different C-terminal domains. The common N-terminal domain contains the *Dot1* homology region including the MT methyltransferase fold required for methylation of histone H3 (FENG *et al.* 2002). Mutation of conserved glycine residues in the active site of both yeast and human DOT1 protein inactivates the enzyme (FENG *et al.* 2002; VAN LEEUWEN *et al.* 2002). GPP also contains domains that are not present in DOT1 including a coiled-coil motif also found in the human, *C. elegans*, *D. pseudoobscura*, and *A. gambia* DOT1-like proteins. In yeast, K79 is mono-, di-, and trimethylated and *Dot1* is responsible for all three modifications (FENG *et al.* 2002; VAN LEEUWEN *et al.* 2002). The different methylated states of H3 at K79 suggest that multiple regulatory activities are conferred on these modified nucleosomes (NG *et al.* 2002; VAN LEEUWEN *et al.* 2002). However, in fly tissue culture cells, the mono- and di- but not the trimethylated form is observed (MCKITTRICK *et al.* 2004). Since database searches indicate that *gpp* is the only fly *Dot1* homolog, it should also be the sole fly protein in this class that methylates histone H3 on K79. Consistent with this suggestion, discs and other tissues isolated from *gpp* mutant larvae have little if any H3 mono- or dimethyl K79.

Like its yeast counterpart, *gpp* is required for the silencing of reporter transgenes inserted into telomeric heterochromatin. However suppression of silencing associated with pericentric heterochromatin is unaffected by mutations in *gpp*. While these observations point to a role of *gpp* in silencing specific for telomeric heterochromatin, our antibody staining experiments indicate that there is a paucity of H3dmeK79 at telomeres in polytene chromosomes compared to many other chromosomal DNA segments. In this respect it is interesting that both telomeric and mating-type chromatin in yeast are hypomethylated on K79 compared to "bulk" chromatin even though DOT1 is required for SIR silencing in each case (NG *et al.* 2003a). It has been suggested that the meK79 modification in euchromatic nucleosomes blocks SIR protein association and that silencing is lost in the absence of DOT1 because the SIR proteins spread into euchromatin (VAN LEEUWEN *et al.* 2002). On the other hand, in flies, since many euchromatic domains in wild-type polytene chromosomes have only little H3meK79, it is difficult to see how telomeric silencing proteins would be restricted to telomeres by this modification even when *gpp* is fully active.

gpp also has functions in flies besides telomeric silencing. Unlike *Dot1*, *gpp* is essential for viability. Although the underlying cause of lethality remains to be established, *gpp* mutant larvae grow more slowly than wild type and this potentially implicates *gpp* in pathways that control growth rates and size in flies. In addition, *gpp* mutants display defects that are characteristic of both *Pc-G* and *trx-G* genes. The first *gpp* alleles were recovered as dominant suppressors of *mini-white* silencing by two *BX-C* PREs. Consistent with a role in *Pc-G* silencing, *gpp* mutants enhance the segmentation defects of several *Pc-G* genes. In this context, it is interesting to note that several *Pc-G* genes have recently been shown to play a role not only in the repression of genes in the homeotic complexes but also in telomeric silencing (BOIVIN *et al.* 2003). Thus, it is possible that *gpp* activity in telomeric silencing may be linked in some manner to its role in *Pc-G* silencing.

gpp mutants also exhibit transformations in segment identity and genetic interactions with *Abd-B* that are characteristic of *trx-G* mutations. This would point to a role in promoting rather than repressing gene expression. Some function in transcription would be consistent with studies in other systems as well as with the enrichment of meK79 seen in many polytene interbands and puffs. However, this correlation is not complete. Thus, there are many puffs and interbands that have only little H3dmeK79. Conversely, H3dmeK79 is sometimes enriched in bands. These findings would argue that in Drosophila, meK79 is not a ubiquitous marker for transcriptionally active chromatin, but rather may have functions that are specific to particular chromatin domains. In this case, the disruptions in homeotic gene expression seen in *gpp* mutants could reflect a special requirement for H3meK79 in the transcription of these particular genes. Domain-specific requirements for *gpp* activity in transcription could also potentially account for the effects of *gpp* mutations on *Pc-G* and telomeric silencing. In this model, *Pc-G* and telomeric silencing would be disrupted in *gpp* mutants because the expression of one or more *Pc-G* (and/or telomeric heterochromatin) genes is downregulated when *gpp* activity is compromised.

The developmental profile of H3dmeK79 also suggests that this modification cannot be a ubiquitous marker for either transcriptionally active or silenced chromatin. High levels of Pol II transcription in somatic nuclei begin in the precellular blastoderm stage around nuclear cycle 11/12. Concomitant with the activation of transcription, H3meK4 can be first be detected at this stage, and the level of meK4 then increases through cellularization (Schaner *et al.* 2003). By contrast, little if any H3 mono- or dimethyl K79 is in either the transcriptionally active somatic nuclei or the transcriptionally quiescent pole cell nuclei. H3meK79 can first be readily detected only later in development in germband extended embryos. However, at this stage accumulation is restricted primarily to a subset of cells in the embryo, most of which seem to be in the process of cell division. High levels of H3meK79 are not observed until stages 13–15, long after the initial upregulation of transcription in the early zygote. This result also suggests that the homeotic transformations seen in *gpp* mutants are unlikely to be due to defects in the initial establishment of parasegment-specific patterns of homeotic gene expression by the gap and pair-rule genes. Rather, these transformations probably reflect a requirement for *gpp* activity later in development during the maintenance phase of homeotic gene regulation—a phase that is dependent upon *Pc-G* and *trx-G* genes. In this respect it is curious that homeotic transformations are not observed in *gpp* embryos when they hatch as first instar larvae. Maternally derived *gpp* activity in homozygous mutant embryos maybe sufficient to maintain specific parasegmental patterns of homeotic gene expression through the end of embryogenesis. Alternatively, there may not be absolute requirement for H3meK79 in maintaining appropriate parasegmental patterns of homeotic expression during embryogenesis.

The developmental profile of H3meK79 indicates that this modification is present at low levels in specific developmental stages and tissues (CNS) undergoing active cell division. In contrast, the highest levels of H3meK79 are observed in epidermal cells that have exited the cell cycle and are undergoing differentiation. Thus, it seems possible that this modification may be activated when specific chromatin configurations, active or inactive, need to be maintained for extended periods of time in the absence of *de novo* DNA synthesis/chromatin assembly. In this respect it is interesting that Mckittrick *et al.* (2004) have reported that the highest levels of meK79 are found in a histone H3 variant, H3.3, which is assembled into chromatin by a replication-independent mechanism. Further studies of *gpp* in Drosophila will be required to understand the mechanisms governing the temporal and tissue-specific regulation of the K79 modification and how this relates to the functions of this particular histone modification during development. Understanding this aspect of the histone code in a multicellular organism such as Drosophila will lead to a better understanding of chromatin regulatory mechanisms during development.

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