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Heterochromatin and gene regulation in Drosophila

Sarah C.R. Elgin
Washington University in St. Louis, selgin@wustl.edu

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We have recently learned more about the biochemistry of heterochromatin and about how heterochromatic environments affect gene function. New findings have emphasized the distinctions between telomeric and pericentric heterochromatin in *Drosophila* and have suggested a mosaic structure within pericentric heterochromatin. Theories concerning the mechanism of inactivation of euchromatic genes in heterochromatic environments have been tested using transgenes inserted into heterochromatin. The current data support a competition/chromatin structure model, in which multiprotein repressor complexes compete with transcriptional activators to assemble an active or inactive chromatin structure.

Address
Washington University, Department of Biology, Box 1229, 1 Brookings Drive, St. Louis, Missouri 63130, USA; e-mail: selgin@biodec.wustl.edu

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Abbreviations

HP1	heterochromatin protein 1
Pc	Polycomb
PEV	position effect variegation
PRE	Polycomb response element

Introduction

The association of heterochromatin formation with gene inactivation

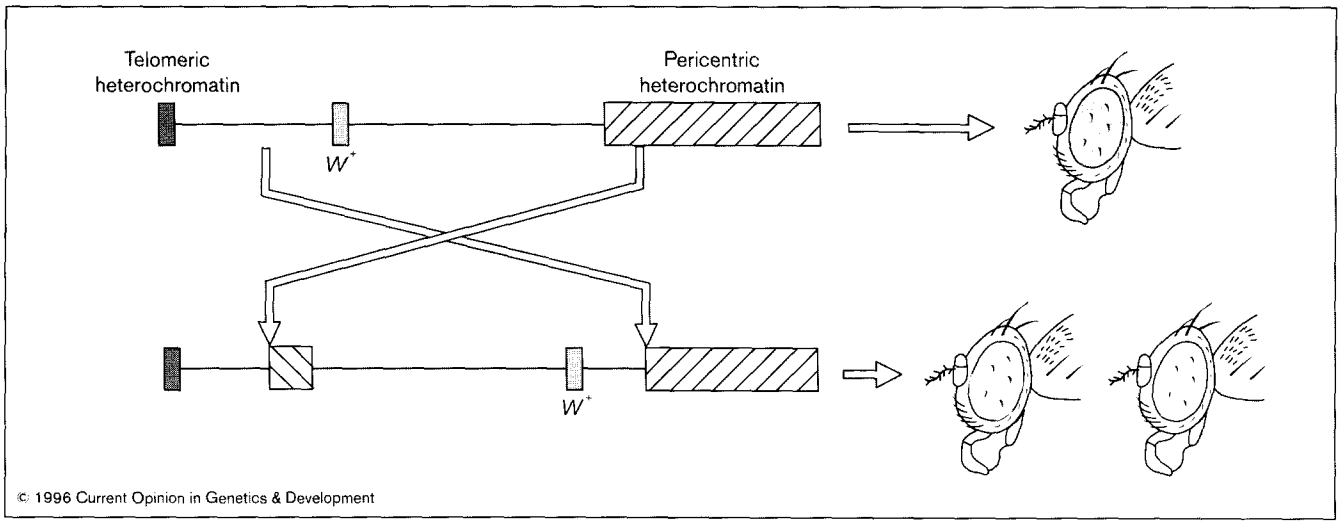
Heterochromatin was originally defined as the genetic material that remains condensed and deeply staining as the cell returns from metaphase to interphase; such material is generally associated with the pericentric regions of chromosomes [1]. With further investigation, the definition of heterochromatin has been expanded to include a broader set of characteristics. The heterochromatic part of a genome tends to be replicated late in S phase [2]. Heterochromatin has been characterized as 'gene poor', although some genetic functions map to these regions. Heterochromatin is made up primarily of repetitive DNA sequences. In *Drosophila*, both highly repetitive satellite DNA and middle repetitive sequences resembling transposable elements are found in the pericentric heterochromatin (see below). Here I will review recent findings on the structure of heterochromatin and on the regulation of genes found in, or near, heterochromatin in *Drosophila*.

Two key observations have linked formation of a condensed heterochromatic structure with gene inactivation. X chromosome inactivation in mammals leaves the inactive X chromosome as a visibly condensed structure, the Barr body. Although the decision as to which X chromosome — maternal or paternal — will be inactivated in the embryo is random in most mammalian species, the decision, once made, appears to be clonally inherited (reviewed in [3]). In *Drosophila*, a similar phenomenon has been associated with the variegating phenotype observed following chromosome rearrangements with one of the breakpoints within heterochromatin. In the example shown in Figure 1, the *white* gene, normally surrounded by euchromatin, is now adjacent to heterochromatin. Expression of this *white* gene is seen in some of the cells in which it is normally active, but not in others; patches of expressing cells are observed, again suggesting a stochastic 'decision' which can be stably inherited through mitosis. While the patterns of gene expression seen with different chromosome rearrangements differ, the hallmark of this phenotype is the presence of two significantly different levels of expression in cells where the gene is normally fully expressed. Because this unusual pattern of expression can be shown to be a function of the novel position of the gene — and not a consequence of any change in the DNA encoding the gene itself — the phenomenon is referred to as position effect variegation (PEV; reviewed in [4,5]). Visual inspection of the polytene chromosomes of larvae carrying a rearrangement of this type has shown that the region of the chromosome including the marker gene is indeed packaged as a dense block of chromatin, but only in those cells in which the gene is inactive. These results indicate a stochastic decision to package the marker gene in a condensed state and a strong correlation between such packaging and gene inactivation [6].

It appears, then, that heterochromatin and euchromatin represent two different structural environments and that these environments have profound effects on gene expression. A similar mechanism for stable gene inactivation has been described in yeast as 'silencing' [7]. Silencing often involves large domains within the genome and is a regulatory process generally independent of promoter/enhancer specificities; most genes are inactivated by this process if they lie within the appropriate domain. Although the on/off state of the gene can be stably inherited, it is reversible. The regulatory process that is 'switched' is probably not the only one operating on the gene; for example, the basal transcription of a test gene in a silent domain can be eliminated although activation by high concentrations of a *trans*-activator is still possible [8••].

Although PEV occurs in response to a chromosomal rearrangement, rather than being a normal part of gene regulation in *Drosophila*, it possesses many of the properties of other silencing systems and provides excellent opportunities for investigation. We would like to know the mechanism by which such decision making is achieved.

Figure 1



A schematic illustration of *white* variegation in the X chromosome inversion *In(1)w^{m4}*. The *white* locus (*w*+) is located in the distal euchromatin (thin line) of the wild-type X chromosome, providing a function essential to the normal red pigmentation of the *Drosophila* eye. The inversion within the X chromosome, *In(1)w^{m4}*, is the result of chromosomal breaks which occurred adjacent to the *white* locus and within the X pericentric heterochromatin (slanted marks). The *white* locus has come to lie 25 kb away from the heterochromatic breakpoint in this inversion. This abnormal juxtaposition gives rise to flies with mottled, or variegated, compound eyes composed of red (*white* gene is active) and white (*white* gene is inactive) eye facets. Patterns vary in the number of pigmented cells, the size of the pigmented patches, and the level of pigment in the two different cell types observed; two examples are shown in the lower part of the figure, one with large patches of pigmented facets and one with pigment in only a few scattered facets. (Adapted from [9].)

What characteristics of heterochromatin are necessary and sufficient to accomplish silencing of euchromatic genes in PEV? How do these relate to the mechanisms of regulation of genes normally present in heterochromatin? How is such a domain-based process triggered? How is the decision maintained through multiple rounds of cell division and how and when is the decision changed? Further, what aspects of this process might be utilized in normal processes in *Drosophila*, for example in the stable inactivation of developmentally regulated genes? As our understanding of the logic of development increases, it has become apparent that maintaining the 'off' state of genes is as critical as maintaining the 'on' state at appropriate times and places (see [9,10] for review). Indeed it has been argued that the ability to maintain a stable off state has been crucial to the evolution of eukaryotes [11].

Three types of hypotheses have been proposed to describe the mechanism of PEV in *Drosophila*. First, it has been suggested that DNA elimination could account for the stable loss of gene expression observed in somatic cells (reviewed in [12]). Second, the visual clues cited above have suggested that differential packaging of the chromatin fiber might account for the differences in gene expression (see [13,14] for models of this type). Third, it has been pointed out that the organization of the genome will influence the location of sequences within the nucleus, and that different spatial subdomains or 'compartments' of the nucleus may provide different opportunities for gene expression, perhaps reflecting local concentrations of enzymes, organizing or condensing factors, and so on [15]. These hypotheses are not mutually exclusive, nor is it necessary that all variegating phenotypes result from the same mechanism.

Heterochromatic genes

A general model for PEV should encompass not only observations on euchromatic genes but also observations on heterochromatic genes. Although heterochromatin in *Drosophila* is 'gene poor', it is not totally devoid of genes. Approximately 40 heterochromatic loci have been identified to date in *Drosophila* (reviewed in [16]). These genes are normally expressed within a heterochromatic environment. Several, including *light*, display a variegating phenotype when subject to rearrangements that change their environment, in this case those placing them adjacent to the distal euchromatin of the X chromosome or of autosomes 2 or 3 [15]. Variegating euchromatic genes are sensitive both to the total amount of heterochromatin in the genome and to mutations in a variety of other loci, presumably encoding chromosomal proteins (see below); variegating heterochromatic genes can show converse responses to these genetic modifiers [17]. Different heterochromatic genes are affected differently by the amount and type of heterochromatin remaining adjacent to them in the euchromatic environment of such rearrangements [18••]. In general, observations on heterochromatic genes suggest that these genes require proximity to appropriate subclasses of heterochromatin to function normally, rather than proximity to the chromocenter *per se* (reviewed in [5]).

This data has suggested that the local nuclear environment or 'compartment' is critical for proper gene expression. Such a compartment might consist of a region of high concentration of certain chromosomal proteins, perhaps maintained by a high density of appropriate DNA-binding sites.

The organization of heterochromatic DNA sequences

For us to understand PEV, we require a knowledge of the DNA sequence organization and protein composition of *Drosophila* heterochromatin. The different satellite DNA sequences are organized in blocks and are present at multiple positions—a map of the relative positions of the different satellites in the centric heterochromatin of the metaphase chromosomes has been established using *in situ* hybridization (summarized in [19••]). Investigation of several transposable elements such as *copia* and *gypsy* (again using *in situ* hybridization to metaphase chromosomes) has shown that each of these sequences is also clustered in one or more discrete regions in the pericentric heterochromatin. These patterns are conserved in geographically distant *Drosophila melanogaster* strains, indicating a stable organization [19••,20•]. At this level of resolution, the patterns of satellite DNA and transposable element DNA overlap, suggesting interspersion. (Note that all of these transposable elements are also present at sites in euchromatin.)

Information at higher resolution has been provided by the use of unique reference sequences, either unique transposons inserted into heterochromatin or unique sequences that are brought adjacent by a breakpoint. An extensive analysis is being carried out of *Dp(1;f)1187*, a stable 1.3 Mb mini-X chromosome that includes a telomere and a centromere, with only a few hundred kilobases of euchromatic material in between. Restriction mapping of rearranged derivatives of *Dp1187* has shown that the pericentric heterochromatin is organized in alternating blocks of complex sequence and satellite DNA, each block being between 60 to several hundred kilobases in length. It is unclear at present whether the blocks of complex DNA, or 'islands', are composed entirely of middle-repetitive transposons, or whether single-copy genes or small amounts of satellite DNA are present within them ([21•] and references cited therein).

A screen designed to detect heterochromatic insertion sites using a *P* element marked with *rosy* recovered a number of insertions into the centric heterochromatin of the autosomes and into the heterochromatic Y chromosome [22••]. Analysis of the DNA surrounding the *P* elements in autosomal heterochromatin has identified sequences of low to moderate repetitive frequency [23••]. Several of the inserts into centric heterochromatin caused lethal mutations that failed to complement a specific heterochromatic deletion and/or could be reverted by *P* element excision, suggesting that these transposons had disrupted essential single-copy genes [22••]. These results are in accordance with the findings obtained by direct cloning and sequencing of heterochromatic genes such as *light*, where the coding region has been found to be unique, but the introns and surrounding sequences are primarily middle repetitive DNA resembling transposable elements [24]. Thus the picture of pericentric heterochromatin DNA sequences that emerges is one that includes unique genes, which are present at a low density, embedded in moderately repetitive transposon-like DNA that is surrounded by blocks of satellite DNA. Different satellites and transposable elements occur in different blocks, creating a mosaic pattern of sequences. This organization no doubt contributes to the differential effects of different blocks of heterochromatin on expression of variegating heterochromatic genes [18••].

Although they are not visibly condensed in interphase cells, the telomeres of *Drosophila* chromosomes show heterochromatic behavior, including the ability to induce a mosaic position effect on genes in the vicinity (reviewed in [25]). *P* element inserts into telomeres also display a variegating phenotype. Again, the recovery of such lines has allowed mapping and sequencing of the surrounding DNA. Telomeric DNA of the right arm of chromosome 3 is composed primarily of two retrotransposons, the HeT-A and TART elements, and their derivatives; other repetitive sequences are also found ([26•] and references therein). It is not clear which sequences are involved in generating the heterochromatic properties of this region (see also Note added in proof). To date complete HeT-A elements have been identified only in DNA cloned from telomeres; related repeats have been found both in telomeric and in pericentric heterochromatin, but not at any euchromatic sites [27•,28]. The HeT-A element functions as a heterochromatic gene, in the sense that it is actively transcribed ([29••]; reviewed in [25]).

The unique *P* element inserts in pericentric heterochromatin described above have allowed a reinvestigation of the process of polytene chromosome formation in *Drosophila*. In this process, the euchromatic arms are amplified 1000-fold, while much of the pericentric heterochromatin remains unamplified and is fused into a common chromocenter. Cytological observations originally suggested that the fused chromocenter contained central α-heterochromatin consisting primarily of satellite DNA, flanked by β-heterochromatin—the unbanded material connecting the chromocenter to the euchromatic arms—made up of middle repetitive sequences. As described above, more recent studies have shown that middle repetitive sequences—transposable elements—are present in blocks throughout the pericentric heterochromatin [19••,20•]. Most of the *rosy*-marked *P* elements in autosomal pericentric heterochromatin described above are flanked by sequences of low or moderate repetition frequency. Nine of these inserts have been tested and found to be fully replicated during polytenization

to levels comparable to euchromatic sequences [23••]. These and earlier results [28] suggest that the β-heterochromatin in *Drosophila* polytene chromosomes is not made up of a contiguous set of sequences — occurring between the satellite DNA of the chromocenter and the euchromatin — but represents an aggregation of the 'islands' of complex, middle repetitive DNA replicated to levels similar to euchromatin during polytenization. The polytene chromocenter would thus be assembled from blocks of under-replicated satellite DNA that associate to make up the α-heterochromatin, interspersed with blocks of fully-replicated middle repetitive DNA, which loop out to form the β-heterochromatin [23••].

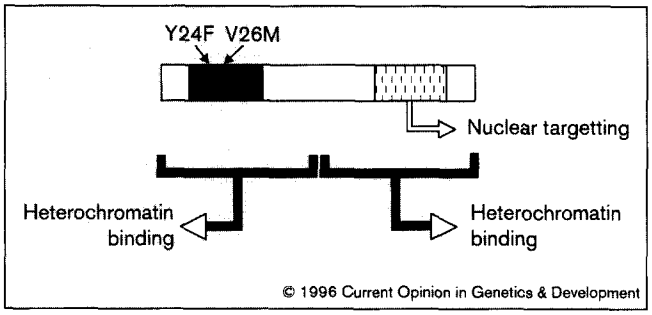
Chromosomal proteins

Over 50 loci have been characterized as suppressors or enhancers of PEV and many more candidates have been identified (reviewed in [5]). These loci, when mutated, result in the increased or decreased expression respectively of a variegating gene. Nearly all are general in action, operating on a variety of genes; ~10% cause both haplo-abnormal and triplo-abnormal phenotypes, causing the increased or decreased expression of the variegating gene when present in one or three copies. Although only a few of these loci have been cloned and their protein products identified, the characterization available has supported the hypothesis (reviewed in [30]) that these genes encode chromosomal proteins or modifiers of chromosomal proteins [31]. Thus PEV — causing gene inactivation — can be suppressed by deficiencies at the histone locus and by mutations in the gene encoding heterochromatin protein 1 (HP1), a protein associated preferentially with heterochromatin. Conversely, PEV can be enhanced by mutations in the gene encoding the GAGA factor, a protein associated with gene activation (see below). These findings in general have been used in formulating models of PEV based on 'switching' gene packaging from a euchromatic to a heterochromatic form [14].

A few suppressors and enhancers of PEV are being studied in detail. HP1 was originally identified in a screen of monoclonal antibodies as a protein primarily concentrated in the pericentric heterochromatin and in a banded pattern over the fourth chromosome [32]. Sequencing of several alleles has clearly shown that HP1 is encoded by *Su(var)205* [33,34], a gene known to cause dosage-dependent shifts in variegation of both euchromatic and heterochromatic genes. (For example, a mutation in one allele for HP1 leads to increased expression of a variegating *white* gene, while three copies or overproduction of HP1 results in decreased expression, the converse response being shown by a variegating *light* gene [17,34].) HP1 is a highly conserved protein; it can be found associated with the pericentric heterochromatin of mammalian chromosomes [35]. The amino-terminal region contains a sequence motif (the 'chromo domain') shared with Polycomb (Pc), a protein involved in maintaining the 'off' state of the homeotic genes. The chromo domain has multiple, but perhaps interrelated, functions in that it is critical for correct localization of the Pc protein — but not of HP1, where a second related 'shadow' domain may be involved — and is critical for gene silencing by both HP1 and Pc ([36,37,38••]; see [39] for review; see Fig. 2).

Neither HP1 nor Pc appear to bind to DNA directly. Several lines of evidence have suggested that the generation of the off state achieved by Pc requires the assembly of a multiprotein complex, including other proteins of the Pc group, defined genetically by their role in silencing homeotic genes. The Pc group proteins can be seen to occupy many of the same sites on the polytene chromosomes and are found in a complex upon immunoprecipitation (reviewed in [10]). HP1 and Pc have very different, almost exclusive, distribution patterns on the polytene chromosomes [9]. However, a chimeric HP1–Pc protein, consisting of the Pc chromo domain replacing that segment in HP1, binds both to Pc sites and to heterochromatin in polytene chromosomes. In *Drosophila* expressing the chimeric protein, endogenous Pc protein is misdirected to the pericentric heterochromatin and endogenous HP1 is mislocalized to Pc sites, suggesting recruitment by protein–protein interactions [38••].

Figure 2



Structure function map of the HP1 protein of *Drosophila*. The horizontal bar represents the HP1 protein. The filled bar represents the chromo domain sequence [64]; the stippled bar represents the chromo shadow domain [65]. Although nuclear targeting activity is restricted to the carboxy-terminal quarter of HP1, both the amino-terminal and carboxy-terminal halves of HP1 have independent heterochromatin-binding activity. Two missense mutations in the chromo domain, Y24F and V26M, that lead to loss of silencing activity in the mutant protein, are indicated above the protein map [38••].

Searches are in progress to identify proteins that might interact with HP1 to generate a multiprotein complex. Most intriguing is the report that the amino-terminal domain of the human lamin B receptor, an inner nuclear membrane protein, interacts with two human homologues of HP1 in a variety of tests (Q Ye and HJ Worman, American Society for Cell Biology 1995, Mol Biol Cell 6:201a). This same domain interacts with *Drosophila*

melanogaster HP1 in a yeast two-hybrid assay (C Shaffer, JA Bernat Jr, SCR Elgin, unpublished data). Analysis of the heterochromatic properties of yeast telomeres has shown that effective silencing, in that case, appears to require localization to the periphery of the yeast nucleus; this positioning requires RAP1, SIR3 and SIR4, the latter two proteins interacting with the silencing domains of histones H3 and H4 ([40••] and references therein).

Overlap between the mechanisms of gene regulation in heterochromatin and euchromatin has been inferred from the observation that some proteins appear to operate as components of both systems. Perhaps the most striking example is the GAGA factor, a DNA binding protein shown to play an important role in the activation of a diverse group of genes (including, but not limited to, homeotic loci), apparently by affecting local chromatin structure (reviewed in [41]). GAGA factor sites have been found to be critical in the promoter region of the heat shock genes for the formation of DNase 1 hypersensitive sites, the gaps in the nucleosome array that allow access for regulatory proteins [42]. In an *in vitro* assembly system, GAGA factor can specify formation of an active chromatin structure at the promoters of heat shock genes [43••]. The GAGA factor is encoded by the *Trithorax-like* gene [44••], which is a member of the trithorax group. These loci play a role in the maintenance of the on state for homeotic loci, working in opposition to the Pc group. However, in addition to its roles in the specific activation of a number of genes and in the maintenance of the active homeotic loci, GAGA factor appears to play a more general role, in that loss of gene function leads to an enhancement of PEV [44••]. Whether or not this activity is based on the same DNA binding required for its role in specific gene activation remains to be seen. This striking result, however, suggests that formation of an activatable chromain structure at the level of the nucleosome array may be necessary, if not sufficient, for packaging a gene in a euchromatic form.

Responses of reporter genes in heterochromatic environments

The heat shock genes, which are generally inactive, are turned on in response to an elevated temperature in almost all cells of the organism and are thus particularly useful in investigations of chromatin structure in different activity states. A screen for heterochromatic inserts has been carried out using a *P* element carrying a *white* reporter gene in a construct that also carries a marked copy of *hsp26* [45••]. Examination by *in situ* hybridization of the variegating lines recovered showed the *P* element to have been inserted into either the centric heterochromatin, the telomeres, or the fourth chromosome in all cases. Insertions within the banded region of the fourth chromosome, as well as in the pericentric and telomeric regions, were recovered. (The small fourth chromosome, which contains ~3% of the *Drosophila* genome [46], has a high concentration of middle repetitive DNA elements distributed throughout [47].) Interestingly, although the variegating transgenes inserted into the pericentric heterochromatin and throughout the fourth chromosome (including its telomere) were found to respond to classical suppressors of PEV, inserts into the telomeric regions of the other autosomes did not respond. Suppression of PEV was seen in the pericentric and fourth chromosome transgenes with increasing heterochromatin dosage — manipulated by varying the number of sex chromosomes — as well as in the presence of *Su(var)205* (a mutation in HP1) and *Su-var(2)1*^{01} (a mutation which results in increased levels of histone acetylation) [45••]. The mutation in HP1 also increased heat shock inducibility of the variegating *hsp26* transgene at pericentric and fourth chromosome sites. These results support a role for chromatin packaging, both at the nucleosome and higher order levels, in causing inactivation of genes in the pericentric heterochromatin and fourth chromosome. Although the variegating phenotype of the *white* transgene at the telomeres was unaffected in the above tests — as reported in earlier studies of transgenes inserted at telomeres (see [5]) — it was affected by the presence of some alleles of *Suppressor of zeste 2* [*Su(z)2*].

Almost all of the variegating stocks recovered in this screen (using a *white* marker) were homozygous viable, indicating that most of the *P* element inserts had not disrupted essential functions. The exceptions were some lines carrying transgenic inserts in the fourth chromosome, a region containing many unique genes [45••]. A comparison of the results of this screen with those described earlier (using a *rosy* marker [22••]) illustrates that the sites of heterochromatic inserts obtained can differ significantly depending on the design of the screen.

Using a rearrangement strategy to place the reporter gene next to heterochromatin, a heat shock-driven *lacZ* transgene has been used to study the timing and stability of silencing by PEV. Again, this reporter can be induced in almost every cell type at any developmental stage. The results obtained, looking at three cases, indicate that silencing begins in embryogenesis, but is suppressed in differentiated tissues; one sees almost complete silencing in the eye discs, followed by a change to an activatable state in some cells of the adult tissue [48•]. This suggests that the silencing seen in PEV is metastable and is relaxed during differentiation. Further studies with different test constructs in different heterochromatic environments will be needed to determine how general this result is; certainly more information of this type is needed to construct reasonable models of PEV.

DNA representation

The genetic studies cited above have provided new opportunities to re-examine the models that have been proposed to explain PEV. To what extent could changes in DNA representation explain the loss of gene function in PEV? In *Drosophila*, both diploid and polytene chromosomes

must be considered. In the past, careful examination of variegating genes has provided some examples in which the gene copy representation in polytene tissues did not change (relative to euchromatic genes) with variegation, and others where it did (reviewed in [49•]). Given the results discussed above on heterochromatic DNA sequences, one might speculate that the cases of PEV where copy representation was maintained involved heterochromatic breakpoints in the complex DNA sequence 'islands', whereas the other cases involved breakpoints in satellite DNA blocks. The variegating genes recovered in the screen [45••] using *white* as a marker have been tested for copy number in both diploid (adult head) and polytene (salivary gland) tissues. No change in gene copy number was found in the diploid tissue. (Note that whereas *white* is expressed in only a few cells, the associated *hsp26* gene, which mimics *white* behaviour in levels of expression from pericentric and fourth chromosome sites, normally can be expressed in almost all cells.) Within polytene nuclei, a severe reduction (33-fold or greater) in copy representation for the heterochromatic transgene, compared to the euchromatic wild-type gene, was observed for the pericentric inserts showing the most extreme phenotype. An approximate two-fold reduction was observed for transgenes in the telomeres and along the fourth chromosome [49•]. The data support the conclusion that DNA loss is not necessary to mediate a PEV phenotype in diploid tissue, but that a decrease in relative copy number could contribute to such a phenotype in polytene tissues. A good example of the latter possibility has been observed for the *Dp1187* minichromosome in XO males. In this case, copy number changes in the *yellow* gene in polytene cells were sufficient to account for the phenotypic variegation observed, although additional effects on transcription could not be ruled out [50]. Thus, although DNA loss may contribute to PEV in special cases, it does not appear to be a necessary component of a general model.

Chromatin structure

The chromatin structure of the variegating *hsp26* transgenes has been investigated using two assays: accessibility to digestion by a restriction enzyme and characterization of the nucleosomal array produced by digestion with micrococcal nuclease [45••]. In the first case, a restriction site within the heat shock regulatory element, known to be accessible in this gene at its normal euchromatic position, was examined. Reduced accessibility was observed in all cases checked, including pericentric, fourth chromosome, and telomeric insertions. The nucleosome array observed for two different *hsp26* transgenes integrated into the pericentric heterochromatin showed a pattern more regular than that observed for the euchromatic gene, suggesting that the transgene might have been forced into an array of regularly spaced nucleosomes [45••]. These results are reminiscent of more detailed findings in yeast. In an extensive study of the silent mating type locus *HMR*a in *Saccharomyces cerevisiae*, a structure that allowed decreased accessibility to restriction enzyme cleavage was observed across a large domain, encompassing the regulatory E and I sites [51••]. In *Schizosaccharomyces pombe*, transgenes in pericentric heterochromatin, which show variegated expression, assume the unusual chromatin structure found in that region [52•]. More data are needed to establish the generality of these observations; the creation of unique sites for mapping within and adjacent to different regions of heterochromatin in *Drosophila*, as described above, should allow considerable analysis of the packaging of these regions and of the packaging effects on inserted transgenes during the next few years.

Several recent reports have indicated that some chromosomal proteins are required both for chromosome function in mitosis and for gene inactivation in heterochromatin, suggesting a common structural requirement, perhaps based on an organizational role of the proteins. HP1, which is critical for PEV, has also been linked to chromosome stability in *Drosophila*; HP1 mutants show defects in mitotic chromosome segregation in the early embryo [53]. In *S. pombe*, it has been found that the *swi6*$^+$ product, a chromo domain protein involved in silencing and concentrated at the silent mating type loci, centromeres, and telomeres, is needed for proper centromere function. Mutations in *swi6* affect transcriptional silencing at the centromere, cause a high frequency of lagging centromeres in late anaphase and have highly increased rates of chromosome loss [54•]. In the nematode, DPY-27, a protein that associates with the X chromosome to down-regulate expression, has been identified as belonging to the SMC family [55], the members of which are generally thought to be involved in chromosome condensation. These findings suggest an overlap in the organizational functions required for packaging metaphase chromosomes and for packaging large domains (in the interphase nucleus) for gene silencing or down-regulation.

Although the GAGA factor is primarily reported to be associated with active genes, it is also seen to colocalize with GA-rich satellite DNAs in diploid cells of the early *Drosophila melanogaster* embryo [56]. GAGA factor is also important for chromosome stability and function in mitosis, perhaps reflecting its association with pericentric DNA [57]. The multiple functions of GAGA factor are reminiscent of those of the Rap 1 protein in yeast, which participates in activation or repression of many specific genes, but is also required to generate the stable structure associated with silencing at the telomeres and contributes to the stable transmission of chromosomes at mitosis (reviewed in [58]).

A competition/chromatin structure model

Recent findings suggest that some components of the mechanism employed to establish active genes in euchromatin—such as GAGA factor—are used to turn on variegating genes in heterochromatin and, perhaps vice versa, that some components of the mechanism employed

to turn off variegating genes in heterochromatin are used to turn off sets of genes in euchromatin (see Note added in proof). The picture is one of competition between establishment of a stable on state, that potentially includes a multiprotein transcription complex and establishment of a multiprotein complex that results in a stable off state. An example of competition of this sort is seen in the regulation of the *URA3* gene placed near a telomere in yeast. Expression of the gene in this location is inhibited at the level of basal transcription, but this inhibition can be reversed by high levels of the appropriate *trans*-activator [8••]. A similar situation has been reported in regulation of a GAL4-inducible gene by the Pc group proteins in a *Drosophila* test system [59••].

Formation of the Pc complex appears to be localized by the presence of a DNA element, the Polycomb response element (PRE). Fly lines were generated containing a construct with a PRE adjacent to the GAL4-inducible reporter gene; as expected, variegated expression of the reporter was observed. Studies using immunofluorescent staining of the polytene chromosomes showed that the Pc and GAL4 proteins had mutually exclusive binding patterns, suggesting that the binding of one precluded the other; induction of high levels of GAL4 eliminated silencing and displaced Pc from the chromosomes [59••]. These results again suggest a competition between a DNA-binding protein, in this case GAL4, and a more complex chromatin structure initiated at an upstream binding site. An intriguing issue that presents itself is whether or not the basic chromatin structure, the nucleosome array, modulates this competition. It has not yet been possible to construct a similar test with an HP1-associated protein complex in *Drosophila*, as the critical sequences for binding — the equivalent of the PRE element, if such exists — have not yet been identified.

A competition model could encompass aspects of both the 'compartment' model and the 'chromatin structure' model of heterochromatin formation. Certainly, the presence of repetitive DNA sequences could lead to both higher local concentrations of some DNA-associated proteins and to greater regularity in nucleosome arrays. At least some satellite DNAs have nucleosome-size repeating sequences, so that the nucleosome packaging which occurs generates a very regular array of uniformly-sized repeating units (e.g. [60]). Uniform nucleosome arrays are not common in chromatin [61]; such arrays might both obscure positive regulatory signals and promote formation of a condensed structure. What is particularly intriguing, in this regard, is the report that multiple tandem copies of a mini-*white* transgene can show variegated expression [62••]. These tandem transgenes are sensitive to classic modifiers of PEV, including mutant alleles of HP1. The effect is stronger for a site near pericentric heterochromatin, suggesting a response to a heterochromatic environment and it strengthened with an increased number of tandem copies. By dictating identical nucleosome arrays, such large repeating sequences might trigger some sort of higher-order packaging that would lead to a stable off state [62••]. This cannot be universal, however, as there are several genes present in tandem arrays — such as histone genes and rRNA genes — that can maintain a highly active state. Again, a competitive balance is suggested.

Conclusions and future prospects

We now have a much clearer picture of the organisation of the pericentric heterochromatin in *Drosophila*; the interspersed blocks of middle repetitive and satellite DNA have different replication and packaging properties in polytene chromosomes and are likely to have different effects in PEV. The second and third chromosome telomeres examined to date, composed predominantly of the retrotransposons HeT-A and TART and related sequences, also behave as heterochromatic domains but respond to different genetic modifiers. Analysis of transgenes in heterochromatic environments has shown that changes in DNA copy number are not necessary to achieve a PEV phenotype in diploid tissue. Alterations in chromatin structure, both changes in accessibility and changes in the nucleosome array, have been observed for variegating genes. GAGA factor can alter nucleosome arrays at specific promoters, creating accessible sites; mutation in the GAGA factor gene enhances PEV, suggesting a possible link between the characteristics of the nucleosome array and the higher order structure of a given gene. A model that encompasses a competitive balance between the assembly of multiprotein complexes that establish the off and on states is consistent with the present data.

The various screens employed to recover heterochromatic transgenes are providing a wealth of material, allowing us to establish the DNA sequence patterns of heterochromatic regions and to evaluate the functional status of test genes at such locations. Further analysis of the chromatin structure and spatial position in the nucleus of such variegating transgenes should allow us to test further the general models proposed for PEV and to design more specific models for testing. The *Drosophila* system allows manipulation both of *trans*-acting and *cis*-acting regulatory elements (the number of copies of a PRE, the level of an activator protein, etc.) in most instances. This system will permit many profitable experiments to be carried out in the next few years.

Note added in proof

In addition to its association with the chromocenter and the fourth chromosome, HP1 is found at a lower concentration in a characteristic pattern of sites in the euchromatic arms and presumably could play a role in silencing in this distinct set of loci. Recently, two human HP1 proteins, hHP1 and hHP2, have been identified as part of a complex that functions in repressing gene expression. These hHPs can repress gene expression in

mammalian cells, but not in yeast cells, when fused to a GAL4 DNA binding domain and tethered to DNA at GAL4 binding sites. Other experiments suggest that hHP1 is part of a complex that silences the human interferon-β gene, a euchromatic gene, in the absence of inducer (M Ptashne and N Lehming, personal communication). These findings suggest that HP1 can also play a role in generating an off state for euchromatic genes, perhaps using a different but overlapping set of partner proteins.

The DNA sequence organisation of the telomere and subterminal region of the left arm of chromosome 2 of *Drosophila melanogaster* has recently been reported. In addition to HeT-A and TART retrotransposons, several kilobases of 457 bp repeat are observed; a single copy region separates this minisatellite from the distal-most gene [*lethal(2)giant-larvae*]. Polymorphisms observed include the insertion of a *roo/B104* transposable element and different arrangements of the HeT-A and TART retrotransposons [63].

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