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HETEROCHROMATIN AND GENE EXPRESSION IN DROSOPHILA

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ABSTRACT

Heterochromatin is both necessary for the expression of heterochromatic genes and inhibitory for the expression of euchromatic genes. These two properties of heterochromatin have been elucidated from the study of chromosome rearrangements that induce position effect variegation (PEV) in *Drosophila melanogaster*. Novel euchromatin-heterochromatin junctions can affect the expression of euchromatic and heterochromatic genes located several megabases away, distinguishing higher order chromatin structure from most other regulatory mechanisms. Studies of PEV promise insights into the basis for heterochromatin formation and the role of higher order chromatin and chromosome structure in gene regulation. We evaluate the models and experimental data that address the mechanisms of PEV in different cell types, the potential functions of modifiers of PEV, and the relationship of PEV to other phenomena associated with variegated gene expression in Drosophila.

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INTRODUCTION

The study of heterochromatin and its relationship to gene expression has a long and rich history that began in the early years of Drosophila genetics. In the 1920s and 1930s, the yearly reports from TH Morgan's laboratory described the discovery of new genetic markers and noted that only a few genes mapped to the Y chromosome, the proximal portion of the X chromosome, or areas surrounding the spindle attachment sites on the autosomes. So few functions mapped to these regions, which were later shown to correspond to the deeply staining, heterochromatic portions of the genome, that heterochromatin became known as the "inert" region (107). However, subsequent observations showed that heterochromatin could affect gene expression. A striking demonstration of the repressive effect of heterochromatin on a euchromatic gene was described by Muller in 1930 (85). He recovered several exceptional mutations of the *white+* (*w+*) gene that resulted in variegated eye color phenotypes. In each case, the mutation was associated with a chromosome rearrangement that placed the gene adjacent to heterochromatin (106). Studies of the *white-mottled* (w^m) alleles and other rearrangements that induced mosaic phenotypes outlined the basic properties of the phenomenon that is now known as position effect variegation (PEV) (72, 114). Early investigators showed that breakpoints that created novel junctions between euchromatin and heterochromatin could induce the mosaic inactivation of euchromatic and heterochromatic genes, genes expressed at diverse developmental stages, and those expressed in different tissues. Position effects induced on a euchromatic gene could be relieved by recombining the gene away from the heterochromatin-euchromatin breakpoint or by inducing a second chromosome rearrangement that changed the location of the gene. Especially intriguing was the observation that a single breakpoint could affect more than one gene, with the strength of the effect diminishing with increasing distance from the breakpoint. The severity and extent of variegation was sensitive to the dose of the Y chromosome and other regions of heterochromatin. Thus, simply changing the balance between heterochromatin and euchromatin in the nucleus could influence gene expression in *trans*.

PEV captured the attention of early geneticists because of the striking phenotypes and because of its potential for understanding chromosome organization and gene expression during cell differentiation (4, 107). The fascination with PEV continues today with the recognition that it provides a genetic approach to investigate how large chromosomal domains are specified and maintained and how chromatin structure influences gene expression (39, 113). This review concentrates on selected aspects of Drosophila heterochromatin and PEV. Certain properties are commonly considered to be defining features of heterochromatin and PEV. We examine the strength of the data upon which

these definitions rest and discuss the implications for understanding the regulation of heterochromatic and euchromatic genes. At the time PEV was first discovered and defined, variegation was a novel phenotype. Since then, a number of phenomena that result in mosaic inactivation of genes have been discovered in a variety of organisms. Variegated phenotypes in Drosophila are associated with repeated transposon arrays, dominant *trans*-inactivation, and position effects induced on transgenes inserted into pericentric heterochromatin, chromosome 4, or telomeric sites. We examine how these phenomena compare to variegated position effects induced in *cis* by chromosome rearrangements. We refer the reader to other references for reviews of genetic functions and molecular organization of heterochromatin (34, 60), or discussions of the relationships of PEV to homeotic gene regulation (25), transvection effects (50), and gene silencing in yeast (1, 61, 89).

CYTOGENETIC AND MOLECULAR ORGANIZATION OF HETEROCHROMATIN

The definition of heterochromatin is based strictly on morphological criteria. Heitz (45) introduced the term to refer to chromosomal regions that appear as deeply staining, compact bodies throughout the cell cycle, including interphase. These regions are distinguishable at the level of light microscopy from euchromatin, which condenses at metaphase, but appears diffuse at interphase. Thus, the definition of heterochromatin was originally, and remains today, limited by the spatial and temporal resolution of a cytological analysis. Even regions generally considered to be "constitutively" heterochromatic may change over time or vary with tissue type, and the boundaries between heterochromatin and euchromatin may not be precisely defined (60). Despite these limitations, heterochromatin is a useful term because it has important implications for chromosome structure and function. The distinction between heterochromatin and euchromatin reflects differences visible at the level of whole chromosomes or large chromosomal domains in the timing or extent of condensation. Heterochromatic regions are generally located at pericentric or telomeric locations (45). They can aggregate together in certain cell types, and thereby promote the association of nonhomologous chromosomes and influence the three-dimensional organization of chromosomes in the nucleus.

The heterochromatin of *D. melanogaster* has been characterized more extensively than that of any other organism (34). In a typical diploid cell, 30–35% of the karyotype is heterochromatic. This includes the entire Y chromosome, 40% of the X chromosome, 25% of chromosomes 2 and 3, and over half of chromosome 4. Heterochromatin is highly enriched in repetitive DNAs (77). The distribution of the major types of repetitive DNAs on the cytogenetic map

of the heterochromatin has been determined by combining chromosome banding techniques and in situ hybridization with repetitive DNA probes (14, 79, 90). The general picture that emerges from these studies is that heterochromatin is molecularly heterogeneous, with specific types of satellite and middle repetitive sequences preferentially clustered at certain chromosomal locations. Lohe and coworkers (79) have estimated that 70–80% of the heterochromatin in diploid cells can be accounted for by 11 different satellite sequences. The molecular dimensions of the simple sequence arrays are estimated to be within the 100- to 900-kb size range, based on long-range molecular mapping (69). The arrays of satellite DNA are interspersed with (69, 90), and may be interrupted by (78), more complex sequences that include different types of middle repetitive DNAs, some of which have homologies to known transposable elements (TEs). Unlike the single TEs found in the euchromatin, heterochromatic clusters of TE-like DNAs show surprisingly little variation in chromosomal location among natural populations of *D. melanogaster*. Hence, middle repetitive DNAs may constitute rather stable structural components of the heterochromatin (90). Single copy DNA sequences from the heterochromatin have also been isolated and in several cases, these are known to correspond to functional genes (8, 11, 19, 20, 35, 88, 131).

Heitz (46) provided early evidence that different regions of heterochromatin have distinctive behaviors and morphologies. He noted that the heterochromatin in salivary gland nuclei coalesces into a single chromocenter and forms two morphologically distinguishable types of heterochromatin, termed α and β. The α heterochromatin was defined as the small compact body in the middle of the chromocenter. Subsequent in situ hybridization studies have shown that α heterochromatin is composed largely of satellite sequences that are greatly underrepresented in salivary gland nuclei relative to other sequences (32). The Y chromosome also appears to be underrepresented as judged by cytological (92) and molecular criteria (134). Heitz (46) defined β heterochromatin as the chromocentral material that is similar to euchromatin in being capable of "growth" and expansion, but that differs in forming a diffuse meshwork instead of well-structured chromomeres. As suggested by Traverse & Pardue (120), β heterochromatin is formed from sequences located throughout the heterochromatin, which replicate to high levels, loop out, then aggregate together to form the bulk of the chromocenter. These sequences include middle repetitive and single-copy sequences located in the heterochromatin of the X chromosome and autosomes (20, 131, 133). At least some β heterochromatic sequences are actively transcribed in salivary gland nuclei, as evidenced by ^{3}H-uridine incorporation (68) and by the detection of RNA transcribed from specific single-copy sequences (8, 19). The functional significance of the differential sequence representation that distinguishes α from β heterochromatin is unknown. Differential representation may be important for regulating the activity

of heterochromatic genes (134) and as discussed below, may also be relevant to the mechanisms causing PEV in polytene tissues.

HETEROCHROMATIC GENES

The relationship between heterochromatin and gene expression is often oversimplified to the extent that "heterochromatinization" is frequently equated with gene repression. While it is true that some regions of Drosophila heterochromatin appear to be largely transcriptionally inactive and induce the inactivation of euchromatic genes in chromosome rearrangements, this is not true of all heterochromatic regions (113). As noted above, heterochromatin also contains transcriptionally active sequences, some of which correspond to genes with vital functions (55).

Approximately 40 heterochromatic loci have been identified thus far in *D. melanogaster* (34). A few of these, including the tandemly arrayed 18+28S rDNAs, and the *ABO* and *DAL* regions that interact with specific euchromatic genes, are repetitive in nature. Thirty genes have been identified by mutations that cause defects in fertility, viability, or morphology. The *light+* (*lt+*) gene, an essential gene located in chromosome 2 heterochromatin, was the first protein-encoding heterochromatic gene to be cloned (19, 20). This gene is expressed in a wide variety of cell types. Its organization differs from that typical of a euchromatic gene. The exonic regions of the *lt* transcription unit are single copy, whereas the flanking regions and largest introns contain a high density of middle repetitive DNAs (19). The repetitive DNAs are heterogeneous and similar to those located in other regions of β heterochromatin (82). Molecular analyses of other heterochromatic loci, such as the *concertina* gene that encodes an α-like subunit of a G protein (88), the *rolled*/ERK-A MAP kinase gene (11; SL Zipursky, unpublished information) and the *kl-5* gene (35) have similar molecular organizations, with repetitive DNAs located near or within the coding regions. The *kl-5* gene, which encodes a testis-specific dynein, is particularly interesting in that it maps to one of the giant Y chromosome loops visible in spermatocyte nuclei (15). The primary transcripts of this and other Y-linked fertility factors are larger than 1 Mb and contain predominantly satellite or middle repetitive sequences (13, 40).

POSITION EFFECT VARIEGATION OF HETEROCHROMATIC GENES

The existence of genetic functions in the heterochromatin raises the question of whether the regulation of some or all of these genes is affected by the surrounding environment. Germline transformation has been used to test this possibility for an isolated 18+28S rDNA coding unit (62). The results showed

that a single rDNA unit inserted into euchromatin is actively transcribed and forms a mini-nucleolus in salivary gland nuclei. Therefore, a heterochromatic location is not necessary for high levels of rDNA transcription, at least in polytene nuclei.

Evidence to support the possibility that some heterochromatic genes require heterochromatin for normal expression was first obtained by Schultz for the *lt+* gene (108). He demonstrated that inversions that remove the gene from the heterochromatin induce its variegated expression. Subsequent studies have shown that PEV of *lt+* is not simply the reverse of PEV of euchromatic genes. Rearrangements that variegate for *lt+* have one breakpoint located between the gene and the centromere, and another breakpoint in the distal euchromatin of the X or autosomes (54, 124). Breakpoints on the Y, chromosome 4, or in heterochromatin or proximal euchromatin of chromosomes 2 or 3 were not recovered in screens for *lt+* variegating alleles.

The conventional view of PEV, based on studies of euchromatic genes, attributes variegation to changes that initiate at the heterochromatin-euchromatin breakpoint and extend to neighboring regions. The breakpoints of rearrangements that induce *lt+* variegation might then be considered extraordinary in having extremely long-range effects that, in some cases, would extend over 5 Mb of DNA (57). A more reasonable explanation proposes that heterochromatin-euchromatin breakpoints cause effects on *lt+* by reducing the amount of heterochromatin that surrounds the gene. This idea is consistent with the observation that smaller blocks of displaced heterochromatin result in lower levels of *lt+* expression (57, 124).

At least five genes near *lt+* also show position effects when displaced from heterochromatin to distal euchromatin, demonstrating that dependence on heterochromatin may be a general feature of autosomal heterochromatic genes (124). Two other heterochromatic genes can be induced to variegate if a sufficient quantity of the surrounding heterochromatin is removed (24). Furthermore, gene activity can be restored by a second rearrangement that places the genes closer to another block of heterochromatin, confirming the idea that the genes require proximity to heterochromatin, rather than proximity to the centromere. Interestingly, heterochromatic genes differ in their requirements for specific heterochromatic regions in *cis* (57). For example, removal of the major blocks of satellite sequences proximal to *lt+* and its neighboring genes results in a severe reduction in activity of some genes. Others show moderate decreases in activity when these sequences are removed, and more extreme reductions as more of the heterochromatin is deleted.

Why do these autosomal heterochromatic genes depend on proximity to heterochromatin for their normal expression? Genetic interactions with modifiers of PEV suggest that this dependence reflects a requirement for the proteins enriched in heterochromatin (39, 44). Increased dosage of the Y chromosome,

which is proposed to compete with other heterochromatic regions for heterochromatin proteins, suppresses PEV of euchromatic genes but enhances *lt+* variegation (5, 106). In addition, twelve genes that act as either suppressors or enhancers of PEV of euchromatic genes have reciprocal effects on PEV of heterochromatic genes (12, 39, 44; C Liep & BT Wakimoto, unpublished information). A model to explain these data proposes that the normal expression of heterochromatic genes requires a critical concentration of heterochromatin proteins that may be present in limiting quantities in the nucleus (124). The repetitive sequences that surround the genes may serve to increase the local concentration of these proteins. Rearrangements that displace heterochromatic genes are expected to decrease their ability to effectively compete for the necessary proteins. The possibility that this competition is influenced by interchromosomal interactions is suggested by the fact that the euchromatic breakpoints that induce *lt+* variegation reside within distal regions of the long chromosome arms. The nonrandom distribution of these breakpoints bears similarities to those that cause transvection effects at other loci. Transvection effects are believed to reflect pairing-dependent interactions between genes on opposite homologs (3, 129). Hence, PEV of heterochromatic genes may be sensitive to pairing because distal euchromatic breakpoints most severely affect the ability of the displaced heterochromatin to associate with heterochromatin on its homologue or heterochromatin on other chromosomes. Variegation may result from the variable ability of the displaced heterochromatin to reside within a nuclear compartment with an appropriately high concentration of heterochromatin proteins. The compartment model, proposed by Wakimoto & Hearn (124), implies an important role for the three-dimensional organization of chromosomes in gene expression (55).

The data above argue against the simple assumption that heterochromatin is incompatible with gene expression. A more realistic view is that heterochromatin and euchromatin represent distinct environments, both of which are permissive for gene activity under the right circumstances. An important goal that remains is to determine if differences exist in the structure of the regulatory regions of euchromatic and heterochromatic genes. These two classes of genes may require distinct types or a different balance of chromosomal proteins to effectively prime a promoter or mediate promoter-enhancer interactions. In this regard, it is interesting to note that Zhang & Spradling (133) reported that transposons inserted within the heterochromatin did not support the expression of a *lacZ* enhancer trap gene. The *lacZ* gene is commonly activated by nearby enhancers when inserted into the euchromatin. However, the gene was not expressed even in those cases in which the transposon caused recessive lethality and was therefore deduced to reside within or near heterochromatic genes. Molecular studies of the *cis*-acting regulatory regions of heterochromatic genes will be important to determine if the surrounding repetitive sequences serve

as transcriptional enhancers and to analyze how the modifiers of PEV affect their expression.

POSITION EFFECT VARIEGATION OF EUCHROMATIC GENES

Models for Repression at a Distance

The demonstration that a breakpoint in heterochromatin could affect the expression of a distantly located euchromatic gene accompanied the first description of PEV by Muller (85). The range of influence of heterochromatin varies among rearrangements (113). In the extreme case of the N^{264-85} translocation, PEV is observed on a gene located 73 polytene chromosome bands (nearly 2 Mb) from the breakpoint (17). How can the influence of heterochromatin extend over such long distances? A consideration of models on the mechanism of PEV sets the framework for addressing this question.

One model that has stood the test of time is the locking molecule model proposed by Zuckerkandl in 1974 (137). He proposed that certain macromolecules, known as locking molecules, are present in the nucleus in limited amounts, and act via repetitive sequences to cause a change from a euchromatic to a more heterochromatic state. As opposed to a simplistic view of chromosomes being divided into two defined states, Zuckerkandl suggested that a "continuous intergradation" exists between extreme heterochromatin and extreme euchromatin. Differences in conformation were proposed to reflect differences in DNA sequence, both in the natural tendency of a region to adopt a higher-order structure and in its affinity for various locking molecules. Histones, enzymes that modify histones, and nonhistone chromosomal proteins were suggested as candidates for locking molecules. The concentrations of these molecules were considered to be as important as the DNA sequence in determining the balance between heterochromatin and euchromatin in the nucleus. The effects of altering this balance by changing Y chromosome dosage led Zuckerkandl to propose that conformational changes respond to the concentrations of locking molecules according to the effect of mass action. The locking molecule model proposes that a heterochromatin-euchromatin breakpoint permits the "creeping" of locking molecules and the coincident propagation of higher-order structure into the euchromatin. The adjacent heterochromatin reduces the threshold requirement for a change from euchromatin to heterochromatin by providing a nucleation site rich in locking molecules. Thus the extent of spreading of the position effect would be determined by the DNA sequence and presence of locking molecule target sites within both the heterochromatin and euchromatin.

Tartof et al (117) proposed a second model to explain the inactivating effects

of heterochromatin on euchromatic genes. According to this boundary model, a heterochromatic domain is defined by the presence of discrete sites that initiate its formation and sites that terminate its linear propagation. The initiator sites were proposed to bind specific proteins that interact with each other in a cooperative manner to generate a compacted heterochromatic domain via polarized spreading. PEV occurs when a heterochromatin-euchromatin breakpoint interrupts this domain. Consequently, a heterochromatic structure is allowed to spread past the breakpoint and proceed into the euchromatin until a site resembling a terminator is reached. Studies of modifiers of PEV led to an elaboration of the boundary model to include a mass action effect (75). The relative concentration of modifier proteins was proposed to influence the extent of heterochromatin assembly.

A comparison of the two models in terms of the initiation, propagation, and termination of heterochromatin reveals mechanistic differences that should be experimentally distinguishable. The major difference concerns how heterochromatin is formed. The boundary model proposes a specific initiator site. The requirements for heterochromatin nucleation are much looser for the locking molecule model, such that one would expect the sequences to have a repetitive component, and potentially a context dependence in activity if isolated from surrounding heterochromatin. Both models share the idea that the inactivating effect of heterochromatin is linear or continuous from the breakpoint. The distance to which the inactivating effect reaches is determined by termination sites in the boundary model. However, in the locking molecule model, the nature of the heterochromatin that nucleates the structural change, and the relative resistance of the euchromatin, strongly affect the extent of inactivation.

One approach to address the question of how heterochromatin is initiated is to identify the sequences that induce PEV of euchromatic genes. Tartof et al (117) asked if heterochromatic sequences located at the breakpoint were sufficient to cause PEV of the *w+* gene. Their study used the w^{m4} inversion, which has one breakpoint ~25 kb from the gene and the second breakpoint in pericentric heterochromatin. Three X-ray induced rearrangements that restored *w+* activity were analyzed. For each chromosome, an undetermined amount of heterochromatin was retained 25 kb from the *w+* gene. The white phenotype appeared to be wild type in these lines, even in the presence of mutations that enhance PEV (75). The authors concluded that variegation had been eliminated in the revertant lines and proposed that an internal heterochromatin initiation site was responsible for the position effect associated with the w^{m4} inversion.

A different conclusion was reached by Reuter et al (100) in a more extensive reversion study of w^{m4}. Of the 51 revertant chromosomes analyzed, the majority were rearrangements that separated the *w+* gene from most or all of the heterochromatin. Some chromosomes restored a completely wild-type pheno-

type except in the presence of enhancers of PEV. Only three chromosomes appeared to be true revertants after this test. Since a molecular characterization of the reversion breakpoints was not undertaken, it is not known if the *w+* gene was completely separated from the heterochromatic sequences in these three chromosomes. However, this study indicated that the retention of some heterochromatin adjacent to the gene was sufficient to cause its inactivation. The authors suggested that heterochromatin is an array of tandemly repeated sequences that show additive effects, an interpretation that is most consistent with the locking molecule model.

Howe et al (57) compared seven translocations that carried a *w+* transgene inserted at the heterochromatin-euchromatin junction to ask if the amount of heterochromatin adjacent to the *w+* gene determined the severity of PEV. The translocations differed only in the size of the heterochromatic block and the nature of the repetitive sequences immediately juxtaposed to the transgene. The strength of the position effect exerted on the transgene differed between translocations and was not correlated with the quantity of the heterochromatin retained at the junction. The boundary model would suggest that *w+* activity was under the influence of a different initiator for each translocation. A second explanation, not exclusive of the first, postulates that only a few initiators exist in the region to which the breakpoints map, but position effects vary with distance from the closest initiator. Fewer suppositions are required if these results are interpreted in terms of the locking molecule model. The stability and type of higher-order conformation assumed by the different repetitive DNAs juxtaposed to the *w+* transgene would determine the degree of variegation.

Is repetitiveness per se responsible for initiating heterochromatin formation? One approach to address the relationship between repetitiveness and mosaic gene inactivation was reported by Dorer & Henikoff (21) using a transposon that carried a minimal *w+* gene. Transposase-induced changes that caused variegated phenotypes were recovered. Unexpectedly, variegation resulted from local amplification of the transposon, with long repeat arrays inducing stronger variegation than short arrays. The relative transposon orientation within arrays also influenced variegation. A relationship between transposon repeat-induced variegation and classical PEV was suggested by the fact that the eye color phenotypes of lines carrying repeat arrays of three or more copies responded to Y chromosome dosage and a mutation that suppresses PEV. The authors proposed that the pairing of multiple copies of the transposon produced folded structures recognized by heterochromatin proteins and that the formation of these structures was influenced by repeat orientation. The Dorer & Henikoff hypothesis that heterochromatin is initiated by folded structures is consistent with Zuckerkandl's locking molecule model. The proposal is similar to an idea expressed by Pontecorvo (91) that heterochromatin is derived simply

from the repetition of sequences. Further experiments are needed to examine the generality of repeat-induced gene inactivation. Recent studies demonstrate that repeated arrays of a transposon carrying the *brown+* (*bw+*) gene do not by themselves result in gene inactivation, although they can enhance variegation of a *bw+* transgene adjacent to heterochromatin (J Sabl & S Henikoff, unpublished information). The relevance of transposon repeat-induced gene inactivation to heterochromatin-induced PEV will be strengthened if the repeat arrays can be shown to induce variegation of a gene that is adjacent to, as opposed to being part of, the repeat arrays.

The idea that gene inactivation spreads linearly from the heterochromatin at the rearrangement breakpoint is a common premise of the locking molecule and boundary models. Indeed, the continuous propagation of heterochromatin is often mistakenly assumed to be a proven character of PEV. However, evidence supporting linear spreading is minimal, with only two reports that address this issue. This is no doubt due to the intrinsic difficulty in proving that linear spreading exists. The expression of at least two genes must be assayed simultaneously in the same cell. The most noted study of the spreading effect is that of Demerec & Slizynska (18), who used a translocation to study the variegation of two genes expressed in the ommatidia. They showed that inactivation of the distal *w+* gene was always accompanied by inactivation of the more proximal *roughest+* gene, while only the *roughest+* gene was inactivated in other cells. Similar results were obtained by Schultz (107) using a rearrangement that caused PEV of the *split+* and *w+* genes. Although these studies permit a local view comparing the effects of heterochromatin on two pairs of genes, they are not revealing with respect to heterochromatin formation between these genes and the euchromatin-heterochromatin junction. Thus, the spreading model is an extrapolation of these data, as it proposes the continuous propagation of heterochromatin from a rearrangement breakpoint through distances that may encompass megabases of DNA.

Are there alternatives to the linear spreading model? It is perhaps easier to imagine how heterochromatin could form at great distances from the euchromatin-heterochromatin junction by envisioning a discontinuous "buttoning" mechanism rather than a "zippering" along the DNA. Such a model is supported by the observations of Belyaeva & Zhimulev (9) on four chromosomal rearrangements that induce variegated phenotypes. They examined polytene chromosome banding morphology near rearrangement breakpoints for evidence of compaction induced by the juxtaposed heterochromatin. In each case, they observed a fraction of nuclei showing bands of normal appearance flanked by regions that appeared compacted. This discontinuous compaction might be consistent with linear spreading if reactivation of some regions occurred subsequent to their initial compaction. Alternatively, a breakpoint that places euchromatin close to heterochromatin may promote compaction at disparate

sites within the euchromatin. Discontinuous heterochromatin formation may also occur in diploid cells. Propagation of heterochromatin could be mediated by chromosome folding, bringing distant regions separated by megabases of DNA into spatial proximity, if not contact (51). Interactions between heterochromatin at the breakpoint and more distant euchromatic regions could serve to nucleate heterochromatin formation at these sites. Thus, the buttoning and linear spreading models for the long-range effects of PEV differ mechanistically in the definition of a heterochromatin initiating site and the role of the novel heterochromatin-euchromatin junction in promoting variegation.

Position Effect Variegation in Polytene Tissues

The unique characteristics of the polytene salivary gland chromosomes have provided important opportunities to study the cytological aspects of PEV and test proposed mechanisms of gene inactivation. Cytogenetic analyses of numerous chromosome rearrangements that induce PEV of euchromatic genes revealed that alterations, such as poor banding, increased staining, and joining with the chromocentral material, are frequently observed in the euchromatin adjacent to a heterochromatic breakpoint (73). This compaction of the euchromatic regions was proposed to result in transcriptional inactivity. However, in some instances, a euchromatic band juxtaposed with heterochromatin became invisible, suggesting a second possibility that heterochromatin induced the variable loss of sequences at the breakpoint.

The most extensive cytological characterization of a region subject to PEV was performed by Zhimulev and coworkers using a translocation that causes variegation of the *ecdysone sensitivity* (*ecs*) gene (136). The *ecs* product regulates the puffing behavior of numerous loci in salivary gland nuclei. Hence, the level of *ecs* expression, as monitored by puff formation, and extent of compaction of the cytological region containing the gene could be monitored in the same cell. In female larvae, the inactive *ecs* gene was associated with compaction of the locus, although compaction was occasionally observed when the gene was active. In male larvae, the gene was almost always active regardless of compaction (135). Therefore, compaction is neither sufficient for, nor strictly correlated with, inactivation of the *ecs* gene. Hence, compaction, gene inactivation, and, potentially, underrepresentation may be independent consequences of chromosome rearrangements in this tissue.

Underrepresentation of DNA sequences in polytene tissue is clearly not a universal cause of PEV. This has been shown for a variegating heat shock locus in salivary glands (49) and for a variegating *rosy+* allele in the fat body and Malpighian tubules (103). Nonetheless, reduced gene dosage in polytene cells might account for PEV in some cases. Variegation of the *Sgs4* gene in salivary glands was associated with a 50% decrease in copy number, a level

that might account for the observed 30% decrease in *Sgs4* mRNA (67). The analysis of a minichromosome that carries a variegating *yellow+* gene showed a gradient of underrepresentation in salivary gland DNA that emanated from the heterochromatin and extended at least 100 kb into the euchromatin (63). However, a correlation between representation and gene expression could not be established since the measurement of gene dosage in the cells that express the *yellow+* gene was not feasible.

The mechanism responsible for the differential representation of sequences within polytene cells is still a subject of debate. The possibility of DNA elimination was first suggested by Schultz (106). Spradling and coworkers proposed that elimination is caused by the activity of transposable elements (63). However, as Henikoff (51) pointed out, the sequences showing the severest levels of underrepresentation in polytene nuclei are the satellite sequences, not the middle repetitive DNAs that are the presumed targets of transposable element transposases. Two other models evoke replication mechanisms. Henikoff (51) suggested a copy choice mechanism in which DNA polymerases switch templates across regions that are ectopically paired because of sequence similarities. This mechanism would result in the preferential underrepresentation of satellite sequences. The simplest and longest standing model for sequence underrepresentation in polytene tissues is underreplication (102). Since heterochromatin is generally replicated later than euchromatin in diploid cells, it follows that late replication may translate to little or no replication during the short endomitotic cycles of polytene cells. Numerous studies have shown that chromosome rearrangements can confer altered replication properties in polytene tissue [for example, see (10, 127)].

DNA underrepresentation is not a likely mechanism to explain PEV in diploid tissues. Indeed, even sequences that show decreased gene dosage in polytene tissue are found to be fully represented in diploid tissue (43, 67; L Wallrath & S Elgin, unpublished information). Moreover, K Ahmad & K Golic (unpublished information) have recently proven that a variegating *white* transgene is present in ommatidia that are phenotypically w–. When FLP-mediated recombination was used to excise the inactivated transgene from the chromosome, the position effect was relieved. Given the considerable differences in the physiology and the balance of heterochromatin and euchromatin in polytene and diploid cells, it would not be surprising to find that different mechanisms can underlie PEV in these two cell types.

Position Effect Variegation and Chromatin Structure

The ability of heterochromatin to induce visible compaction of juxtaposed euchromatic bands in polytene chromosomes is often viewed as supportive evidence for the chromatin condensation model of PEV (39). According to

this model, PEV is caused by structural alterations in the chromatin assembled at the heterochromatin-euchromatin breakpoint and beyond. Large multimeric complexes of heterochromatin proteins are proposed to assemble onto the adjoining euchromatin, forming a molecularly compact structure that is inaccessible to transcriptional machinery.

The sensitivity of PEV to conditions suspected to affect histones provided indirect evidence that variegation might involve changes in chromatin structure. The variegation of several different euchromatic genes is suppressed by chromosomal deficiencies of the histone locus (83) and by treatments with butyrate (84), or carnitine (29), compounds that inhibit histone deacetylation. These compounds also cause lethality in flies that are homozygous for *Su(var)2–1*^{01}, a suppressor of PEV associated with a recessive hyperacetylation of histone H4 (22, 29, 95). These data are consistent with the idea that histone dosage or specific histone modifications may differentially affect heterochromatin and euchromatin. Turner et al (123) recently showed that acetylated isoforms of histone H4 differ in their distribution within euchromatin and heterochromatin in salivary gland chromosomes. β-heterochromatin is depleted in H4 acetylated at either lysine 5 or 8 but enriched in H4 acetylated at lysine 12.

Only a few studies have directly addressed the question of whether PEV is correlated with changes at the level of chromatin structure. Three of the four studies used the w^{m4} inversion to compare the chromatin accessibility of rearranged and nonrearranged sequences. The accessibility of a fragment that contained the *w+* gene to DNase I digestion was assayed in embryonic and larval nuclei by Hayashi et al (42). Locke (74) monitored the resistance of the heterochromatin-euchromatin junction fragment to degradation by endogenous endonucleases using nuclei isolated from adult heads. Schlossherr et al (105) applied a particularly sensitive, quantitative method to measure the accessibility of restriction endonuclease sites in the *w+* promoter using nuclei isolated from adult eyes. As measured by these three assays, the w^{m4} inversion did not induce significant effects on chromatin structure. This was true even in genetic backgrounds that caused severe repression of the *w+* gene (42, 74, 105) and strong effects on the compaction of the *w+* locus in salivary gland chromosomes (42).

A correlation between PEV and altered chromatin structure has recently been established in an important study by Wallrath & Elgin (125). The gene monitored in this case was a transgene containing the *hsp26* promoter fused to heterologous sequences. Transgenes inserted into heterochromatin showed reduced levels of expression after heat shock induction compared to a euchromatic control transgene. Importantly, a specific restriction site located upstream of the *hsp26* promoter showed decreased accessibility. The overall nucleosomal organization of transgenes located at two different heterochro-

matic locations was also monitored using micrococcal nuclease digestion. In both cases, the transgenes were organized into a more ordered nucleosomal array when compared to control insertions. Thus, this study demonstrated for the first time, using two different assays, that heterochromatin-induced gene inactivation correlates with a change in chromatin structure.

The contrasting results of the *hsp26* and the w^{m4} studies may reflect differences in the sensitivity of the techniques or in the mechanisms inducing PEV. The chromatin structure of the *hsp26* promoter has been extensively studied (126) so the position of a critical nucleosome could be precisely monitored. In contrast, the chromatin conformations of the active vs inactive states of the *w+* gene have not been characterized, and if differences exist, they may be less distinct than those that occur at the *hsp26* promoter. It is also possible that the w^{m4} rearrangement alters a higher level of chromatin or chromosome organization that is not easily monitored by nuclease sensitivity (42, 105). For example, Schlossherr et al (105) proposed that variegation of the w^{m4} allele may result from its decreased ability to access subnuclear compartments containing a high concentration of transcription factors, a mechanism similar to that proposed for PEV of heterochromatic genes (124). Clearly, additional studies are needed to examine whether changes in chromatin accessibility are characteristic of only some variegating alleles. These studies will require a judicious choice of promoter and assay conditions to maximize the chances of detecting what may be subtle differences in chromatin conformation.

MODIFIERS OF POSITION EFFECT VARIEGATION

A systematic genetic approach to identify *trans*-acting proteins that affect the severity of PEV has been conducted primarily in the laboratories of Grigliatti and Reuter. Large-scale screens for mutations that act as dominant suppressors (*Su(var)*s) or enhancers (*E(var)*s) of the w^{m4} phenotype have been carried out, with the aim of recovering genes that encode proteins involved in the assembly or maintenance of chromatin. Hundreds of modifiers, representing at least 50 loci, have been identified thus far (39, 98). Nearly all these modifiers have proven to be general in action since they affect the expression of several different variegating alleles.

The dominant modifiers of PEV are characterized by dosage sensitivity. The vast majority are haplo-abnormal, either suppressing or enhancing PEV when present in a single dose. A small proportion, estimated to be 10% (98), causes both haplo- and triplo-abnormal phenotypes. Examples of this second class include the *Su(var)205* and *Su(var)3–7* loci. A deletion of either gene suppresses PEV of w^{m4} (96, 111) while increasing gene dosage to three or more copies or inducing overexpression enhances PEV (28, 97). Genes with these

reciprocal modifier effects have been postulated to encode structural components of the heterochromatin or euchromatin (75).

The molecular analysis of the gene encoding HP-1 (59) and the discovery that it corresponds to the *Su(var)205* gene (27) was particularly satisfying since the protein is localized predominantly to the chromocenter of salivary gland nuclei (58). HP-1 is also present throughout chromosome 4, at telomeric sites, and, although less prominent, at multiple euchromatic sites. The localization in salivary gland nuclei is compatible with the idea that HP-1 is primarily a structural component of heterochromatin. However, a study of the protein in dividing cells reveals a more complex situation. In early embryos and tissue culture cells, HP-1 is enriched in the heterochromatin, but it is also present throughout the interphase nucleus (66). During metaphase and anaphase, a significant fraction of the protein is no longer associated with condensed chromosomes, but is dispersed throughout the spindle. HP-1 becomes associated with chromosomes again at telophase. This dynamic pattern of binding and the presence of multiple phosphorylated forms of the protein (26) suggests that cell cycle regulation may be important for determining HP-1 distribution in the genome. HP-1 may be important not only for gene expression (25, 44) but also for proper chromosome segregation. Kellum & Alberts (65) demonstrated that embryos with reduced quantities of HP-1 show an extension in the duration of prophase and defects in chromosome morphology and segregation.

Genetic and molecular analyses of other modifier loci indicate that they constitute a group of genes with diverse functions. For example, the *mus209* gene is associated with recessive mutant effects that include suppression of PEV, mutagen sensitivity, female sterility, and reduced viability. This gene encodes the Drosophila homologue of PCNA, the proliferating cell nuclear antigen essential for DNA replication (47). The *Su(var)3–6* gene, a haplo-abnormal dominant modifier, is associated with recessive mitotic defects and encodes PP1, the catalytic subunit of a type I protein phosphatase (6). Several other modifier genes encode putative protein products with motifs suggestive of DNA binding or protein-protein interactions. The *Su(var)3–7* gene encodes a putative Zn-finger protein with acidic stretches similar to those characteristic of members of the HMG group of nonhistone chromosomal proteins (97). The *modulo* (*mod*) gene, a dominant haplo-abnormal suppressor, encodes a DNA-binding protein that localizes to heterochromatin and >100 euchromatic sites in salivary gland nuclei (33). The expression patterns of the *mod* protein during embryogenesis and the pleiotropic effects of *mod* mutants have suggested that the protein may regulate genes required for the morphogenesis of specific tissues (38).

The collection of genes identified as modifiers of PEV overlaps, although not extensively (31, 36), with genes that regulate homeotic gene expression (98). This overlap strengthens the hypothesis that related molecular mecha-

nisms may underlie PEV and homeotic gene regulation, an idea first suggested by the discovery that the HP-1 and Polycomb proteins share a common protein motif known as the chromodomain (25). Perhaps the most intriguing member of the class of genes that function in both PEV and homeosis is the *Trithorax-like* gene (30). This gene was identified independently as a positive regulator of homeotic genes, as an enhancer of PEV, and as the gene that encodes the GAGA protein, a transcription factor that binds GA/CT-rich sites near a variety of different promoters (7). The GAGA protein facilitates transcription of the heat-shock genes by changing chromatin structure and the arrangement of nucleosomes at the promoter (80, 122). The simplest model proposes that the protein plays a similar role in homeosis and PEV, but this remains to be tested. The GAGA protein binds to a large number of euchromatic sites in salivary gland chromosomes (122). Interestingly, it is a conspicuous component of heterochromatin in diploid cells, colocalizing with the large blocks of AAGAG and AAGAGAG satellite repeats (93). It is not known whether this localization in heterochromatin is functionally important, either for heterochromatin formation or for modulating the levels of the GAGA protein available for binding to euchromatic sequences. The latter possibility implies that the normal expression of euchromatic genes may be affected by quantitative or qualitative changes in the heterochromatin. This type of interaction has been suggested to explain the dosage-sensitive interactions between the *ABO* heterochromatic loci and the euchromatic *abo* gene. In this case, *ABO* heterochromatin might titrate out factors that negatively regulate the expression of the *abo* gene or the abundance of its product (115, 118).

It has been tempting to try to fit all of the modifier loci into a common framework, with each affecting PEV via mechanisms that affect chromatin structure. The validity of this framework will continue to be tested as additional genes are cloned and the functions and targets of their products are elucidated. The pleiotropy associated with many modifier mutations may reflect multiple functions of their protein products. For example, modifier proteins that act on PEV and homeotic genes, and those that bind euchromatin and heterochromatin, may carry out these different activities via distinct mechanisms. The Rap1 protein of *Saccharomyces cerevisiae* provides an interesting paradigm for considering this idea (110). This protein binds repetitive sequences at the telomere. It also acts as either a positive or negative transcriptional regulator, depending on chromosomal context. DNA binding, activator, and repressor activities are mediated by distinct protein domains, with each dependent upon different protein-protein or protein-DNA interactions. The putative protein product of the *Su(var)3–9* gene is a candidate for a similar type of complexity (121). This protein combines a chromodomain with a SET domain, a motif shared with *E(z)*, an enhancer of transvection effects, and *trithorax*, a positive regulator of homeotic genes. Context-dependent interactions may also explain

how some modifiers of PEV, including *Su(var)205*, can have opposite effects on the variegation of euchromatic genes and heterochromatic genes (44).

The number of modifiers of PEV is unexpectedly large. Reuter estimates that about 120 genes modify PEV, based on the prevalence and distribution of dominant *Su(var)*s and *E(var)*s (98). However, the number is likely to be considerably higher since the genome has not been saturated with dominant modifiers and a systematic search for recessive modifiers has not been performed. Therefore, a practical goal in the analysis of modifiers is to identify criteria for discriminating between those informative for understanding the mechanism of PEV and those that simply reflect the sensitivity of PEV to nonspecific perturbations. Potentially useful criteria for classifying the modifiers include determining which most strongly affect heterochromatic gene expression (44) or *trans*-inactivation (116). These assays may identify proteins that act preferentially on specific heterochromatic regions or proteins involved in pairing interactions between heterochromatic regions or homologous chromosomes. Thus far, only a few modifiers have been tested for their effects on chromosome stability (6, 65, 128), polytene chromosome morphology (42, 99), and chromatin structure (42, 74, 105, 125). Additional data are needed to determine if modifiers generally affect these processes. Genetic tests to assess the extent of overlap between the collection of genes identified as modifiers of PEV and those identified on the basis of other phenotypes can also provide potentially useful information for understanding the mechanism of action of modifier proteins. For example, the existence of a few genes that participate in PEV, homeosis, and perhaps also transvection has suggested a mechanistic link between these processes (25, 98, 130). Another example has been provided by Birchler and coworkers. Two genes initially identified as dosage sensitive regulators of the *w* gene also suppress PEV of euchromatic genes (12, 16). Both genes also affect the steady state levels of retrotransposon RNAs, suggesting the interesting possibility that some modifiers may affect PEV by altering the expression of transposable elements (12).

TRANS-INACTIVATION

PEV is commonly thought of as a recessive effect, confined to the gene on a rearranged chromosome. Indeed, only *cis*-inactivation is observed for the vast majority of cases. However, variegating alleles of three genes, *brown+* (*bw+*) (85), *Punch+* (86), and *karmoisin+* (48) are dominant, causing *trans*-inactivation of the nonrearranged allele on the homologue. The most extensive genetic characterization of dominant PEV has been conducted for the *bw+* gene (112). Recent experiments by Henikoff and coworkers have shown that the dominance of *bw+* PEV requires an interaction between alleles that is sensitive to chromosome pairing (52).

Sequences that mediate dominant PEV of *bw+* have been investigated using transgenes that can be induced to variegate by chromosome rearrangements. A deletion analysis of a *bw+* transgene in *cis* to heterochromatin revealed an inverse correlation between deletion size and ability to *trans*-inactivate the *bw+* transgene on the homologue. Dreesen et al (23) suggested that physical contact between the heterochromatin on the rearranged chromosome and the transgene on the homologue was suppressed by the looping out of sequences caused by the deletions. Similar results were obtained from a deletion analysis of the transgene on the nonrearranged chromosome. However, *trans*-inactivation in this case was most sensitive to deletions of the 5′ region of the gene, suggesting the existence of an upstream element that is sensitive to heterochromatin. These data support a model in which expression of *bw+* gene requires a transcription factor whose activity or binding to 5′ regulatory sequences is decreased in the presence of heterochromatin proteins (23). Any model to explain the mechanism of dominant PEV must explain why this phenomenon is so rare.

Studies of dominant PEV of the *bw+* gene have revealed similarities to PEV of heterochromatic genes. Chromosome rearrangements can suppress dominant PEV of *bw+* by either disrupting pairing between rearranged and nonrearranged alleles (52) or moving the heterochromatin that is inducing the effect beyond a certain distance from the pericentric heterochromatin of the long autosomes (53). These features suggest that formation of heterochromatin at the variegating breakpoint depends upon its proximity and perhaps its physical association with other heterochromatic regions in the nucleus (51).

CHROMOSOME 4-INDUCED POSITION EFFECT VARIEGATION

Chromosome 4 is the smallest chromosome of the Drosophila complement. At least half of chromosome 4 is generally considered heterochromatic on the basis of staining properties (56). The remaining portion appears well banded in salivary gland chromosomes and contains an abundance of middle repetitive sequences (82). Interestingly, regions dispersed throughout this chromosome are capable of inducing variegation of euchromatic genes via chromosome rearrangements (41) or transposon insertion (125). Position effects exerted on transgenes inserted into the well-banded portions of chromosome 4 are similar to those induced by pericentric heterochromatin in being suppressed by the Y chromosome and *Su(var)* mutations (125). In addition, at least one chromosome 4 transgene shows the reduction in chromatin accessibility that is characteristic of insertions in pericentric heterochromatin.

Genetic and cytogenetic analyses of chromosome 4 indicate there is a frequent interspersion of regions that contain functional genes and sequences

that induce PEV of euchromatic genes (56). This organization suggests two possibilities for the relationship between genes and their neighboring sequences: the genes depend on the sequences capable of inducing PEV of euchromatic genes in a manner similar to that proposed for the chromosome 2 heterochromatic genes (124); or alternatively, the genes may be protected, perhaps by boundary elements (101), from the inhibitory effects of nearby sequences. The overall molecular organization of chromosome 4 may be similar, in some ways, to the transition zones that exist between heterochromatin and euchromatin of the larger chromosomes (82). The sequences, perhaps the middle repetitive DNAs, that induce PEV and confer properties of heterochromatin, may change gradually from proximal to distal regions along the long chromosome arms, forming a series of intermediate states with euchromatin at one extreme (137). If so, some of the repeated sequences in the euchromatin of the X, chromosomes 2 or 3 might be expected to induce variegated position effects. Data consistent with this idea have been obtained by Ahmad & Golic (unpublished information). Insertions of a *w* transgene into proximal euchromatin resulted in variegated eye color phenotypes that were suppressible by the Y chromosome.

TELOMERIC POSITION EFFECT VARIEGATION

The telomeres of *D. melanogaster* are not heterochromatic by cytological criteria. However, they can induce variegated position effects. Transgenes inserted into telomeric regions have provided convenient tools for studying telomeric position effects (TPE). The position of the transgenes with respect to telomeric sequences has been characterized for an insertion at the tip of the right arm of chromosome 3 (3R) and for a set of insertions on the Dp1187 minichromosome. A variegating *w+* gene is inserted ~16 kb away from the 3R terminus, within a series of tandem, 1-kb repeats (70, 71). These repeats are proximal to a region of interspersed TART and HeT-A elements, two retroposons important for telomere maintenance (81). Twenty-six transposons that show variegated expression of the *ry+* gene are inserted into a 5-kb region located ~ 40 kb from the Dp1187 terminus (64). This region is composed of a tandem array of repeats (64) that are related in sequence to the 1-kb repeats that flank the *w+* transposon (R Levis, unpublished information).

Sequences that induce PEV of the *w+* gene have been mapped by analyzing partial revertant lines showing increased *w+* expression. These lines were associated with terminal deletions, the loss of sequences distal to the transposon (70). A clear correlation was observed between a red eye color and the loss of a portion of the 1-kb repeats, and a yellow eye color and the retention of some of the repeat region (109). An analysis of the variegating *ry+* telomeric insertions showed that terminal deletions also relieved these position effects

(119). Thus, a specific type of repetitive DNA into which transgene is inserted may be primarily responsible for inducing the position effect. This can be verified by determining if the 1-kb repeats are sufficient to induce variegation at nontelomeric sites. Interestingly, Tower et al (119) reported that one variegating *ry+* transgene was inserted into a HeT-A element adjacent to the P element hotspot.

As a general rule, modifiers of PEV do not affect TPE. The variegation of the *w+* transgene is affected very little, if at all, by Y chromosome dosage or by the action of a large number of genic modifiers of PEV (R Levis, unpublished information, 116). Six different *hsp-w+* transgenes located at chromosome 2 or 3 telomeric sites are not affected by Y chromosome dosage or two *Su(var)* mutations (125). However, exceptions to this rule are observed. Variegation of the *ry+* transgenes inserted on Dp1187 is suppressed by the Y chromosome (64) and three *hsp-w+* chromosome 4 telomeric inserts are suppressed by two *Su(var)* mutations (125). The sequences inducing PEV of the chromosome 4 transgenes are not known.

How can the differential effects of modifiers of PEV on these telomeric transgenes be reconciled? Differences may reflect the chromosome- and strain-specific variations that exist in the identity and organization of telomere-associated repeats (81). Variegation of the Dp1187 and chromosome 4 transgenes may be induced by repetitive DNA sequences that are present not only at the telomere, but also within heterochromatin (64, 132). These transgenes may then respond to modifiers of PEV if the telomeric and heterochromatic repeats compete for heterochromatin binding proteins. Alternatively, sensitivity to modifiers of PEV may depend upon the extent of the repeated sequence array. The Dp1187 telomere is a minimum of 45 kb (64), whereas the 3R telomere in the variegating *w+* strain is less than 27 kb (71). At this point, the existing data do not permit a valid comparison between telomere size (i.e. the distance between the last gene and the terminus) and character.

An especially attractive model to explain the differential sensitivity of TPEs to modifiers postulates that chromosome size is an important factor (116, 125). The transgenes showing modifiable repression are inserted on a minichromosome, which is only 1.3 Mb (64), or on chromosome 4, which is ~5 Mb (76). In salivary gland nuclei, the minichromosome is indistinguishable from the chromocenter (63) and both ends of chromosome 4 show a unique association with the chromocenter (56). Perhaps this association with other regions of heterochromatin increases the competitive ability of telomere-associated repeats, having only moderate intrinsic affinity, for heterochromatin proteins. This model can be tested by determining if chromosome rearrangements that change the distance between telomeric transgenes and pericentric heterochromatin alter the sensitivity of TPE to modifiers.

The absence of a consistent effect of modifiers of PEV on chromosomes 2

and 3 TPEs indicates that mechanistic differences exist between these position effects and those induced by heterochromatin. Support for this idea has been obtained from studies comparing the chromatin structure of transgenes inserted into regions causing variegation of an adjacent marker gene. Wallrath & Elgin (125) quantitated the accessibility of a restriction endonuclease site in the promoter region of the uninduced *hsp26* transgene and in all cases found it to be reduced, when compared to an insertion into euchromatin. In contrast, inducibility of *hsp26* by heat shock was inhibited for pericentric heterochromatic and chromosome 4 insertions, but not for the chromosome 2 and 3 telomeric inserts. In these cases, induction of transcription from the *hsp26* promoter could overcome the altered chromatin state imposed by the telomere. This property is reminiscent of TPE in *S. cerevisiae* (37). When the *URA3* gene is placed adjacent to a telomere, its expression is inhibited in a subpopulation of the cells. This inhibition is overcome if the *URA3* gene is induced. Interestingly, a strain that is mutant for the *PPR1* gene, which encodes an activator of the *URA3* gene, shows orders of magnitude greater sensitivity to telomeric repression (94). Further studies of *URA3* repression indicated that silencing by the telomere acts at the level of basal transcription, and illustrated the antagonistic role of *trans*-activators in TPE (2).

TPE in Drosophila and *S. cerevisiae* may have more in common with each other than with PEV induced by pericentric heterochromatin. Extensive studies of yeast TPE have identified the *cis*-acting repetitive sequence that induces gene silencing and the principal *trans*-acting factors (104). Recent studies have also suggested a potential role for localization of the telomeres to the nuclear periphery in gene silencing (87). The relatively small size and complexity of Drosophila telomeres should facilitate analyses of the *cis*-acting sequences that induce TPE in this organism. The identification of mutations that suppress or enhance TPE should provide a particularly profitable avenue for determining if novel proteins exist that recognize specific types of telomere-associated repetitive sequences.

SUMMARY AND PERSPECTIVES

Studies of Drosophila heterochromatin argue against the simple view of heterochromatin as a molecularly compact structure that is inaccessible to the transcriptional machinery. Indeed, numerous studies have revealed that the heterochromatin in this organism is heterogeneous at the molecular and genetic levels. The existence of heterochromatic genes, some of which require proximity to heterochromatin, suggests that a more interesting relationship exists between heterochromatin and gene expression. We have emphasized the idea that heterochromatin and euchromatin are distinct environments, each compatible with the expression of certain types of genes. Differences in these two

domains exist in overall sequence organization and the constellation of binding proteins. The number and diversity of proteins involved in specifying, maintaining and remodeling these domains may be quite large. While some proteins preferentially recognize specific types or arrangements of heterochromatic sequences, others are common to both heterochromatin and euchromatin. Proteins in this second class may function in general aspects of chromatin structure or transcription. Shifting the balance of these proteins in the nucleus, by altering the heterochromatic content or changing the levels of specific proteins, is expected to have consequences for the expression of both heterochromatic and euchromatic genes. We consider PEV to be a particularly sensitive assay for revealing these effects. We would not be surprised to find that heterochromatin plays a more significant role in the normal regulation of euchromatic genes than is generally perceived.

The very features of heterochromatin and PEV that make them fascinating biological phenomena also make them challenging to investigate at the molecular level. Nonetheless, the application of diverse cytogenetic, genetic, and molecular strategies has led to significant advances in our understanding of the structure and complexity of heterochromatin. The molecular analysis of modifiers of PEV will continue to yield important information about the *trans*-acting proteins that distinguish heterochromatin from euchromatin. The progress made in recent years in tagging and cloning heterochromatic regions has set the stage for future studies of the DNA sequences that are required to form heterochromatin, support the expression of heterochromatic genes, or induce inactivation of euchromatic genes. The functions of specific types or arrangements of repetitive sequences isolated from different heterochromatic regions can be compared using germline transformation.

Elucidating the molecular basis for the long-range effects of heterochromatin on gene expression remains an extremely important goal and a major challenge. We and others have emphasized that multiple mechanisms may account for position effects induced by chromosome rearrangements. The models proposed for the mechanisms of PEV have provided useful experimental frameworks for studying aspects of heterochromatin that include its sequence organization and its relationship to gene expression, chromatin condensation, and nuclear organization. We anticipate that studies of Drosophila heterochromatin and PEV will continue to yield insights not only into the issue of how large chromosomal domains are specified and maintained but also into other fundamental issues of chromosome structure and function.

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