24. Position-effect Variegation in *Drosophila*

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I. Introduction and Prologue

A discussion of position-effect variegation should start with a definition. It is the mosaic expression of a gene lying near a breakpoint in a chromo-

some rearrangement. Mosaic expression is most easily demonstrable for cell-autonomous phenotypes displayed in large numbers of similar cells in essentially two-dimensional array—the hypoderm, the ommatidia—but can be inferred in other instances. Most loci examined have been found to be susceptible to variegated expression. The mosaicism is in expression, the gene being active in some cells but inactive in others; there are a number of lines of evidence, to be discussed, that render unlikely an accompanying mosaicism in genetic coding due to mutation.

Variegation is evoked chiefly by rearrangements in which one or both breakpoints lie in heterochromatin. Sometimes several neighboring loci whose actions can be detected in the same cells are near a variegation-evoking breakpoint. Their combined pattern of expression indicates that the portion of chromosome inactivated has linear contiguity with the point of breakage though its extent varies from cell to cell. The clonal pattern of expression in many instances indicates that the extent of the inactivated region is determined rather early in development and maintained with considerable faithfulness through many subsequent chromosome replications and cell divisions.

The phenomenon was first described by Muller (1930) under the label "eversporting displacements". He ascribed it to either chromosomal or gene instability or to an effect on gene action by an abnormal chromosomal position, perhaps through interaction of local gene products. Dobzhansky (1936) included the "eversporting displacements" with cases like Bar in his review of position effects, on the grounds that whatever the ultimate explanation of these mosaic phenotypes, they were invariably associated with rearrangement breakpoints. A number of other proposed mechanisms —impairment of gene function in structural heterozygotes (Noujdin, 1944; Ephrussi and Sutton, 1944) or disruption of the nucleic acid metabolism of the chromosome (Schultz, 1956)—were advanced. The work prior to 1950 was very clearly reviewed by Lewis (1950). Accepting Dobzhansky's classification of "eversporting displacements" as position effects, Lewis introduced the distinction between stable and variegated position effects. Hannah (1951) and Baker (1968) have provided more recent summaries of information about variegated position effects.

As to the mechanism of position-effect variegation, little is yet known with certainty. More than forty years of speculation on the subject have led to several hypotheses which have individually generated numerous experiments, many interesting observations, and, usually, rebuttal of the tested hypotheses. I shall not present here a history of fallen hypotheses. However, there are certain factors whose effects on variegation beg for explanation.

Temperature at critical stages of development, amount of certain types

of heterochromatin in the genome, parental source of the rearrangement, and several specific loci are all modifiers of the extent of variegation for a large number of rearrangements. The effects of these factors and the properties of "heterochromatic" regions, as they are presently beginning to be understood, together suggest that an understanding of the mechanism may be near at hand.

II. The Chromosomal Geography of Variegation Induction

A. Cis-dominance and the "Proof of Position Effect"

Only genes in the rearranged chromosome variegate. It was first pointed out by Baker (1968) that a cis–trans relationship exists between the variegating gene (g^+) and the breakpoint (bp): $g^+ \; bp/g \; bp^+$ shows mosaic expression of g^+—patches of g amid patches of g^+ tissue—while only the usual phenotype associated with the heterozygote g^+/g is uniformly displayed by the recombinant $g^+ \; bp^+/g \; bp$. The exceptions to cis-dominance are probably only apparent. Those few cases of "dominant" variegation—where $++/g \; bp$ seems to variegate—seem best explained as ordinary cis-dominant effects on adjacent loci whose function is essential to the apparently variegating "trans" locus. Specific evidence for this will be discussed in Section II, E.

Cis-dominance is the basis of the "proofs" of the position-effect nature of the variegation.

1. Crossing Over between Locus and Breakpoint

In three variegation-inducing translocations, crossovers have been obtained between the affected locus and the breakpoint. The crossover exchanged the variegating allele from the rearrangement for a recessive mutant from a normal-sequence chromosome. The resulting translocation chromosome behaved as if it carried a simple recessive. In two cases the previously variegating allele was recovered in the normal-sequence chromosome and behaved as the typical dominant wild-type allele, proving that its variegation had resulted from its situation near the breakpoint.

Panshin (1935) replaced the variegating allele occupying the *cu* locus in $T(3;4)Dl^{?P}$ with the mutant *cu*, the breakpoint being 0·8 map units to its right. The allele recovered from the translocation was a fully dominant cu^+. When the mutant in the translocation was again replaced by cu^+, from an unrelated stock, the original level of variegation was resumed. Dubinin and Sidorov (1935) had similar results from a similar two-step substitution of a new h^+ into $T(3;4)684$.

Judd (1955) found recombination at a rate of 0·1% between white and

the breakpoint of $T(1;4)w^{m258-21}$ to the right of *dm* (3·1 map units from *w*). A typical w^+ was recovered in two normal-sequence recombinants. With *w* in the translocation, *T*(*w*), no variegation was evident regardless of the allele in the normal X, though other loci such as split continued to variegate. $T(w^a)/w$ displayed apricot-white mosaicism, while $T(w^a)/+$ appeared wild-type.

2. *Further Rearrangements Separating Locus and Breakpoint*

In several studies, a variegating locus has been separated by later rearrangement from its variegation-inducing breakpoint. A new level of variegation is established, depending on the new location. When the new location is the same as or generally similar to the normal location for the locus, normal wild-type action is resumed. Nearly exact reinversions, restoring the wild-type phenotype, have been induced in *In(2LR)40d* (Hinton, 1950) and $In(1)rst^3$ (Grüneberg, 1937; Novitski, 1961). Panshin (1938) found that the white mottling associated with $T(1;4)w^{m11}$ was reduced or eliminated when the w^m-containing tip of the X was moved to euchromatin. More extreme variegation resulted from an additional heterochromatic breakpoint near the white locus in the derived rearrangement.

B. Loci Subject to Position-effect Variegation

1. *Euchromatic Loci*

I shall call "euchromatic" those loci placed in salivary chromosome sections 1–19, 21–39, 42–60, 61–79, 82–100. Of these loci, very few have not been found to variegate in appropriate rearrangements upon a direct search. The first and most commonly recurring case is white-mottling. With 12 pigment cells per ommatidium and more than 800 ommatidia per eye, very fine-grain mosaicism is detectable (Gersh, 1952).

Variegation detected as obvious phenotypic mosaicism has been found for all autonomous sex-linked loci and most of the autonomous autosomal loci affecting ommatidial pigment or arrangement and repetitive hypoderm (cuticular) structures that have been screened. This includes loci once thought to be heterochromatic because of rarely recombining with the centromere, such as *in* (at 77BC) and p^p (85A–D). Some of the non-autonomous loci may reveal mosaicism in future screening. With appropriate histochemical methods variegation for loci affecting enzyme activity can be detected. With these methods, variegation for loci such as *mal* (which lacks aldehyde oxidase) could be detected. Mosaicism for *mal* due to chromosome loss has already been demonstrated (Janning, 1972). Even the salivary glands can be mosaic for puffs—for example the 3D puff in

$T(1;4)w^{m258-18}$ (Schultz, 1965). Only one locus has so far not yielded variegants upon search—*e* (Brosseau, 1970), although *e* is autonomous in chimaeric tissues produced from imaginal disk cell mixture or somatic crossing over.

Non-autonomous loci, or loci whose "point" mutants have quantitatively variable expression, can be inferred to variegate when the rearranged chromosome shows "weakened dominance"—some degree of mutant expression—in heterozygotes with hypomorph or amorph alleles. One such locus, hairy, was the first to be used in the crossover demonstration that it was the position of the wild-type allele in the rearrangement that caused its partial mutant expression (Section I, A.1). For three loci—*Pgd*, *Amy* and *v* (tryptophan pyrolase)—flies have less of the enzyme coded by the allele in the rearrangement (Bahn, 1971; Tobler *et al.*, 1971; Gerazimova *et al.*, 1972).

Factors such as extra Y chromosomes that reduce or eliminate mutant patches in most of the mosaic variegation systems usually also reduce mutant expression in these cases of "weakened dominance". One test that has been applied in searching for variegation of loci with such drastically all-or-none phenotypes as lethality is the comparison of X0 with XY and with XYY. By this method, variegation has been identified for lethals scattered throughout the X chromosome (Lindsley *et al.*, 1960; Ben Zeev and Falk, 1966). Such loci have been more precisely localized to the right of *sc* (Baker, 1971), to the right of $su(w^a)$ (Rayle and Green, personal communication), to *dor* (Lucchesi and Bischoff, personal communication), in the zeste-white interval (Kaufman, 1970; Judd *et al.*, 1972), to the right of white (Lefevre and Green, 1972), and near *ras* and near *v* (Barr, personal communication). Ben Zeev and Falk (1966) reported failure to induce Y-suppressed lethals on the second chromosome, but since they apparently scored. near-lethality (few homozygous survivors) as non-lethals, or at least did not compare percent survival of X0 with XY, they may have missed some lethal-variegation.

Lefevre (1972) has pointed out that the high proportion of loci essential for life found in the three, presumably representative, small chromosome regions that have been studied intensively probably accounts for the scarcity of XO-viable position-effect evoking inversions, and the localization of those that do occur to the regions of apparent repeats—1B1–4, 3C, 15F–16A.

2. *Heterochromatic Loci*

I shall call "quasi-heterochromatic" those loci whose location in salivary gland chromosomes is in the polytenized parts of regions 20, 40–41, 80–81 or 101 at the base of each chromosome arm, extending from the

chromocenter (cf. Lefevre, Chapter 2 Vol. 1a). In dividing cells, chromosome regions are considered to be heterochromatic if they are condensed during the major part of interphase. This condensation persists into prophase during which the euchromatic regions gradually condense, permitting recognition of the extent and location of the heterochromatic parts of chromosomes. Most of the chromosome regions so identified in larval ganglion cells or gonia are unreplicated or underreplicated during polytenization of the salivary gland chromosomes (Rudkin, 1965, 1969) and chromosome deficiencies for major fractions of the heterochromatin seen on a ganglion cell chromosome are not visible in salivary gland chromosomes (Hinton, 1942; Baker, 1954; Schalet and Lefevre, 1973; Lefevre, Chapter 2). The traditionally "heterochromatic" sections at the bases of the chromosome arms include parts that are replicated, perhaps parts that are underreplicated, and parts that are not replicated at all. At least the parts replicated in salivary gland chromosomes appear euchromatic in mitotic chromosomes (Hinton, 1942; Rudkin, 1965). These replicated basal sections have been called heterochromatic because of their highly irregular appearance, with bands diffuse and difficult to distinguish, varying from cell to cell, reflecting either an irregularity in the condensation of chromomeres in the individual chromonemata of the polytene chromosome or a lack of uniqueness in the inter-strand associations formed by these chromomeres (Prokofyeva-Belgovskaya, 1947; Viinikka *et al.*, 1971; Hannah-Alava, personal communication).

I shall reserve the term "heterochromatic" for those loci in the non-polytenized regions proximal to the polytene quasi-heterochromatic sections. They are usually indistinguishable in the chromocenter, the ectopically-paired association of the centromeric regions of all the chromosomes in the salivary gland nuclei. In some species of *Drosophila*, but not *melanogaster*, whole metacentric chromosomes often pull away from the chromocenter. The unreplicated centromeric region is represented by a constriction (Yoon, personal communication).

Some loci probably non-replicated in the salivary gland chromosomes have been induced to variegate. These include peach, located distally in the region between the most proximal band of chromosome 5 and its centromere in *D. virilis* (Baker, 1953), the male fertility factors on the Y chromosome of *D. melanogaster* (Neuhaus, 1939; Benner, 1970) and *D. hydei* (Hess, 1970), and light (*lt*), which is proximal to the banded parts of section 40 (Hessler, 1958).

Variegation of an underreplicated region has been found for the nucleolus organizer region (Baker, 1971; Hannah-Alava, 1971). The nucleolus organizer region is underreplicated in salivary gland chromosomes, at least in *D. hydei* (Hennig and Meer, 1971). Its position in the salivary

gland X chromosome of *D. melanogaster* has been debated. Although Hannah-Alava (1971) has most recently placed it in 20 C or D, it now seems that at least 10 non-complementing ordinary loci including *su(f)* can be assigned to the bands of section 20 (Schalet and Singer, 1971; Schalet and Lefevre, 1973). All of the bands of section 20 are included in inversions such as sc^4 having proximal breaks to the left of the nucleolus organizer (Cooper, 1959; Schalet and Lefevre, Ch. 21). The nucleolus organizer is thus proximal to the whole of the polytene section 20.

The *ci* locus is probably in replicated salivary gland chromosome quasi-heterochromatin. It has been placed by Hochman (1971) at the boundary between 101 and 102, in one of the bands missing in $Df(4)M^{63a}$ (101F–102 A1,2). Its variegation has been extensively studied, and shares many of the properties of the heterochromatic loci listed above.

C. Location of Variegation-inducing Regions

1. *Inducers of Variegation of Euchromatic Loci*

Almost all rearrangements inducing euchromatic loci to variegate juxtapose these loci to centromeric heterochromatin. In most cases the effective heterochromatin is the part proximal to the break, although some variegation results from the distally relocated portion. Not all heterochromatic regions are equally effective; crude distinctions can be made between the metacentric autosomal arm bases and the base of the 4th chromosome; between the regions proximal to and distal to the nucleolus organizer on the X; and between the regions distal to the male fertility factors and the remainder of the Y chromosome.

Both the "heterochromatic" autosome bases (sections 40, 41, 80, 81 and 101) and the non-polytenized regions of the salivary gland chromosomes are effective. For example, consider the centromere region of chromosome 3. Two different insertions of nearly the same segment of the X—$Dp(1;3)N^{264-58}$ and $Dp(1;3)w^{m49a}$—have markedly different degrees of variegation for vital loci near the boundaries of the inserted X segment (Ratty, 1954). The bands of the first duplication are visible clearly between 80C and 80D. The second is at the base of 81, is less clearly visible in the salivary gland chromosome, and "covers" lethals much less well. On the other hand, not all breaks in 80 evoke variegation. One of the reversions of the variegation associated with 26D loci juxtaposed to 41A (in *In(2LR)40d*) occurred when region 26D was subsequently translocated to 80C (Hinton, 1950).

When a sizeable length of euchromatin is inserted into an autosome arm base, either in the replicated or non-replicated portion, the loci nearest

the boundaries of the inserted segment may variegate while those toward the middle are unaffected (Demerec, 1941). Even for the distally located $Dp(1;3)N^{264-58}$, the distalmost locus, *dm*, is more severely affected—appearing fully mutant except under conditions that strongly suppress variegation (Spofford, 1973)—than the loci immediately proximal to *dm*. The long insertion of 3C1–6A1,2 just proximal to 102A in $T(1;4)N^{264-85}$ does not normally variegate for loci in 3F through 4C (Demerec, 1940). The w^+ locus does not variegate in $T(1;2)w^{62g26}$, an insertion of 2E1–4A1 into the non-polytenized part of chromosome 2 (Lefevre, 1970). Thus both the distal and proximal portions of the interrupted heterochromatin are variegation-inducers.

Rather "small" portions of heterochromatin, removed from their usual centromeric position, can occasionally cause variegation. This is the only reasonable inference from the studies in which variegating rearrangements have been subjected to further rearrangement. Often partial reversions are accompanied by detachment of the affected loci from their position in the first arrangement and relocation in a euchromatic region—but with further intense variegation in a subsequent location, also euchromatic. Presumably the new breaks were not induced at precisely the same positions as the old, and some of the variegation-inducing heterochromatin, whose presence in a polytene chromosome would be detected only as a constriction if at all, was moved along with the marker locus (Panshin, 1938; Griffen and Stone, 1940a; Kaufmann, 1942). The variably banding element associated with bw^D has been similarly interpreted as a heterochromatic insertion (Slatis, 1955b). The other, rather rare, instance of variegating phenotypes not associated with heterochromatin detectable in the salivary gland chromosomes may have a similar explanation.

It is possible that the short left arm of the 4 is also variegation inducing. Both w^{m11} (Panshin, 1938) and w^{mA} (Griffen and Stone, 1940b) are metacentric with complete complements of 4th chromosome loci in one arm and regions 1–3C in the other. An alternative explanation of w^{mA} is that the translocation occurred in an iso-4, since the purported 4L in the illustration accompanying the report appears to be a reverse repeat of the basal section of 4R.

The heterochromatin of the sex chromosomes, although a major fraction of the total heterochromatin of a mitotically dividing cell, is comparatively less frequently involved in variegating rearrangements than is autosomal heterochromatin. Thus, among 8 translocations giving *bw* variegation induced in one study, none involved the X chromosome, though they would have been readily detected (Slatis, 1955a). Presumably more than half of the rearrangements juxtaposing X or Y heterochromatin to euchromatin do not disturb the functioning of the euchromatic loci. It seems reasonable

either to ascribe this to heterogeneity within the heterochromatin, variegation-inducing regions interspersed among non-inducing regions, or to a lesser spread of the variegation induced by X-chromosome heterochromatin.

As for the X, it is not yet clear whether breaks in section 20 are variegation inducing. Many of the earlier localizations to 20 were relative to a location for the nucleolus-organizing region now considered incorrect. Those based on observation of salivary gland chromosome cytology are suspect because of the extreme variability of banding in region 20. The actual breakpoint may lie anywhere between the reported position in 20 and either *bb* (if noted as to the left of the latter) or the centromere. Reversions of variegation have been obtained for rst^3 at 18F and 19E (Kaufmann, 1942) and for w^{m11} in 20 (Panshin, 1938). Inversions with the same breakpoint near *su*(w^a) evoke variegation when the other breakpoint is in the non-polytenized region between *su*(*f*) and *bb* but not when it is in 19E (Rayle and Green, personal communication).

Both the non-polytenized regions to the left and to the right of the nucleolus organizer are effective. To the left, embracing the region proposed by Lindsley (1965) as critical to inactivation of the entire X during spermatogenesis, are the effective breaks for rst^3, w^{m4}, w^{mJ}, B^{M1}, m^K and sc^4, to list a few of the variegating X inversions. In some of these the variegation-inducing region remains near the centromere and the affected euchromatic loci are brought near it (rst^3, w^{m4}). In the others, the variegation-inducing region is removed from the centromere and brought near the distally located affected locus. In the case of *In*(*1*)rst^3 it is clear that the break occurred within the variegation-inducing region, since the distal *w* locus also variegates in an X0 male (Gersh, 1963). In the case of the inversion with the most distal heterochromatic break (Cooper, 1959), *In*(*1*)sc^4, there is no evidence for variegation induced in the loci (especially *l*(*1*)*sc*) brought proximally. Again, breaks to the right of the nucleolus organizer can divide the variegation-inducing region. In sc^8, the proximal *sc* and *l*(*1*)*sc* loci and the distal *ac* locus all variegate (Raffel and Muller, 1940; Baker, 1971). In sc^{V2}, the proximal *sc* and the distal *l*(*1*)*J1* both variegate (Baker, 1971).

The nucleolus organizer, together with an undetermined extent of heterochromatin to either side of it, can induce variegation in its new neighbors when transposed into euchromatic surroundings, for example, near *ct*, *lz* or *in* (cited in Hannah-Alava, 1971).

The short right arm of the X induces extreme variegation of the 3C3–6 region in *In*(*1LR*) *l–v 139* (Gersh, 1965), and has a strong effect on *ac* in *In*(*1LR*) sc^{V1}.

The picture that emerges for the centromeric portion of the *D. melanogaster* X chromosome is of a transition from the typical euchromatin

(organized into chromomeres that cohere homologously into the bands of polytene chromosomes) of sections 1–19 through a region of irregular polytenization and chromomere condensation and of dubious capacity to evoke variegation (section 20) to a region whose condensation in most interphase cells is not fine-grained (yielding chromomeres) but instead very coarse (yielding heterochromatin). The distal section (Cooper's hD), broken in sc^4, is separated from the more proximal sections by a region that is less likely to condense at certain phases of the cell cycle (hence, yielding a visible constriction) and is perhaps less likely to induce variegation. The nucleolus organizer is not itself heterochromatic by the usual criteria of condensation and non-transcription, but is adjoined on both sides by regions which are; in at least parts of both of these regions breaks are variegation-inducing.

Again, only parts of the Y chromosome induce variegation. Translocations to the Y have been shown to be the cause of some instances of variegation of *w*, *N*, *v*, *dp* and *bw*. However, the locations of breaks on the Y that induce variegation in euchromatic loci have rarely been studied. Tobler *et al.* (1971) reported that the variegating v^+ in a $T(1;Y)y^+ Yv^+$ was distal to the KS male fertility loci, but with an indeterminate extent of Y^L distal to the v^+ locus. On the other hand, some of the reversions induced in *In(2LR)40d* resulted from relocating the variegating loci from region 41 to the Y chromosome (Hinton, 1950). Thus, the property of inducing variegation is apparently not uniformly distributed along the considerable extent of sex chromosome heterochromatin, though there is no present reason to doubt its uniformity in the shorter heterochromatic regions at the bases of the autosome arms.

2. *Inducers of Variegation of Heterochromatic Loci*

Variegation of *ci* (the "Dubinin effect") and *lt* in *D. melanogaster*, of *pe* in *D. virilis*, and of the male fertility factors in both *D. melanogaster* and *D. hydei*—identified in the latter as suppression of specific loops—is evoked most readily by breaks separating these loci from their centromeres and moving them somewhere into the distal three-quarters of the euchromatic arms. Of 193 rearrangements giving variegation of *ci* analysed by Khvostova (1939, 1941), over twice as many involved chromosome 3 (114) as chromosome 2 (47). None of the variegation-inducing breaks occurred in the proximal euchromatic regions of the long autosomes, although some occurred in 41, 80 and 81 when these latter were removed from the centromere by additional rearrangements. Only 12 were on the X, but the entire length of the salivary gland X, including section 20, was

variegation-inducing. Breaks in or distal to the fertility-factor region of Y^S and distal to at least *kl-2* in Y^L generate ci^+ variegation (Neuhaus, 1939; Parker, 1967; Benner, 1970). Benner found that even a portion of Y^L distal to *kl-2*, but not including an entire active *kl-2*, region could continue to evoke *ci* variegation so long as the original Y-4 junction was not severed during detachment of a compound X chromosome. Probably few of the types of new junction formed at the base of the X could themselves evoke *ci* variegation since only one was found among many detachments of *T(Y;4)*'s with full ci^+ expression. Stern and Kodani (1955) found that breaks in the centromeric as well as distal regions of the long autosomes induced "dominant" variegation in mutant *ci* chromosomes, the probable basis for which will be discussed in Section III.E. Similarly, Hessler (1958) found *lt*-variegation-inducing regions to be restricted to the distal or very centric portions of the X, 2 and 3, in a smaller series of rearrangements. Ganglion mitotic figures confirmed that substantial portions of the 2L heterochromatin, including its secondary constriction, had been moved distally.

In *D. virilis*, Baker (1953) induced pe^+ variegation by translocation to distal euchromatic locations in other chromosomes or to the Y chromosome either proximal or distal to, but not within, the male fertility region. Some pe^m mutants placed the distal portion of the heterochromatin of chromosome 4 distal to the *pe* locus without removing *pe* from the centromere of 5.

Variegation of the male fertility factors is the most probable reason for Neuhaus' (1939) finding that 38 out of 46 induced *T(Y;4)* ci^V's were male sterile. Complementation testing indicated the inactive *kl* or *ks* factors to be those translocated to section 101. Presumably many translocations had been induced in the same large-scale study with breaks proximal to the fertility factors—but were undetected because basal Y and basal 4 heterochromatin provided similar conditions permitting normal action of the *ci* locus. More recently, Benner (1970) analysed a number of Parker's *T(Y;4)*'s with a break in Y^L that had been isolated without regard to *ci* effects. Several had relatively low activity of all the *kl* factors remaining attached to the Y centromere, giving low fertility in combination with tester-Y chromosomes. The activity of these factors varied with temperature in the manner typical for position-effect variegation. The variegation, if such it was, must have been induced in this case by the distally placed 4R, itself broken to the left of (i.e. proximal to) ci^+. One ci^+ fragment lacked *kl-2* activity entirely, but in further rearrangements with the basal X regained *kl-2* activity and simultaneously began to variegate for *ci*.

In *D. hydei*, Hess (1970a) recovered several instances of variegation of

certain of the Y lampbrush loops in translocations to the basal heterochromatin of XL, which is short in metaphase chromosomes in *D. hydei*, the major part of the X heterochromatin and the nucleolus organizer region being in XR. The effective section of the X may correspond to region hD in the *D. melanogaster* X chromosome.

The nucleolus organizer has been shown to variegate when moved by inversion to near the tip of the X. Baker (1971) inferred this from the lethality associated with $In(1)sc^{S1}$ and $In(1)sc^{L8}$ that persisted in variegation-enhancing genotypes (e.g. XO) when all possibly variegating euchromatic loci were covered by duplications. Nix (1973) confirmed the deficit of rRNA in $sc^{S1}/0$ first instar larvae. Hannah-Alava (1971) and Breughel (1970, in *D. hydei*) both report extreme variability in the salivary gland chromosome cytological appearance of the nucleolus in transpositions to euchromatin. Sometimes the nucleolus appeared merely as a puff. However, it must be noted that the appearance of the nucleolus is extraordinarily variable even in its normal location.

In summary, certain loci normally located in or very near heterochromatin can be induced to variegate when repositioned either at a considerable distance from heterochromatin or within certain other heterochromatic regions. Except for the X chromosome, the more proximal euchromatin does not evoke variegation.

D. Relation of Breakpoint to Affected Loci

1. *Effective Distance*

How near to a variegation-inducing breakpoint must a locus be to be affected? The answer seems to depend on the particular variegation-inducing region, the particular euchromatic region, and perhaps even the direction from the locus in which the breakpoint lies.

There are several instances of notably great distances. Among these are the 50-band interval between bi^V (4C9–4D3) and the break to the left of 3C5 in $In(1)N^{264-52}$, the 35-band interval between cx^V (in or to the left of 5B), and the break to the right of 6A2 in $Dp(1;4)N^{264-85}$ (Demerec, 1940), the 67-band interval between the most distally affected band, 2B14, and the break to the right of 3E5 in $T(1;4)w^{m258-21}$ (Hartmann-Goldstein, 1967), and especially the 80-band interval between ac^V (1B1–2) and the breakpoint in 3A in $Dp(1;f)R$. The first of these, an X inversion, is cited as having its right break between 20B and C, which can best be interpreted as meaning "somewhere to the right of 20B". The next two include an insertion and a reciprocal translocation in the part of the fourth

chromosome that is often broken but represented in the salivary gland as a single interband between 101F and 102A1. The last again involves a break in basal X chromosome heterochromatin, but in a chromosome retaining the reverse repeat of the heterochromatin through section 20A1 on the other side of the centromere of the original chromosome, a ring.

Other instances of long distance propagation of the variegation effect are afforded in other species. In *D. virilis* (Baker, 1954), the *pe* locus is distal within the basal heterochromatin of chromosome 5. Two $T(3;5)pe^{m}$, numbers *4* and *53*, show only a small amount of translocated heterochromatin in the pe^{m}-carrying element in ganglion prometaphases. However, $T(3;5)pe^{m51}$ has a break at the extreme proximal end of the heterochromatin, which comprises almost half the length of the normal chromosome 5. Similar cases exist in *D. hydei*. For example, a break leaving the proximal quarter of Y^{L} attached to Y^{S} nevertheless led to inactivation of the noose-forming site in Y^{S} in most males (Hess, 1970a).

On the other hand, the effect often does not extend for more than a very few bands and may not even extend to the band adjacent to the break except under conditions that are generally strongly enhancing, for example white mottling in $In(1)rst^{3}$ occurs only in X0 males.

Variegation induced by a Y chromosome breakpoint is often closely localized. For example, $su(w^{a})$ (1D4–1E1–2) is included in the very extensive region of variegation in *Dp(1;f)R*. Rayle and Green (personal communication) studied a series of 4 $su(w^{a})^{V}$ and 3 $su(w^{a})^{+}$ *Dp(1; Y)*'s formed from an $XY^{L} \cdot Y^{S}$ chromosome, with right hand breakpoints to the right of *su(f)* and thus either in what basal X heterochromatin remained in the compound XY or in the Y distal to the KL region, or more proximally in the single $su(w^{a})^{V}$ *Dp(1;f)* recovered. Strong variegation accompanied a break in the 1E3–4 doublet. Weak variegation sometimes but not always accompanied a break separating the doublet from 1E5. No break separating 1E1–2 from 1E3–4 was recovered giving a variegated phenotype, perhaps because such close proximity inactivates $su(w^{a})^{+}$ in all rather than only part of the eye tissue.

2. *The Spreading Effect*

Whenever the affected locus is several bands from the nearest break, what of the intervening loci? Can the inactivation process skip over them? An answer requires a judicious choice of test situations. If a hemizygous viable inversion or homozygous viable translocation were found with one or more essential ("lethal") loci between the breakpoint and 3C2, with striking white locus variegation in eye, testis sheath and Malpighian

tubule in live adults, it would not mean that those lethal loci were not variegating. It would mean that their normal action was not essential to the development of the eventually pigmentless cell-lines in eye, testis sheath and Malpighian tubule after the time that the chromosomal basis for inaction of the white locus was established. Considerable cell death may have occurred in those tissues to which normal action of these loci is essential.

In point of fact, as Hannah pointed out in 1951, no viable X-chromosome inversion giving a position-effect on white has a breakpoint to the right of 3C5—the lethality associated with inaction of the entire 3C3–6 region (Lefevre and Green, 1972) must apparently be expressed in all or some of these tissues.

A proper assay system then requires a tissue in which both affected loci normally act at approximately the same time in the same cells, or at least in the same cell lineage, during a period in which the physiological basis of gene inactivation is not altered. Such a condition is realized in the eye for many pigment and facet arrangement loci, or in the hypoderm for bristle shape and pigment loci, when the variegation is coarse grained (large-patch) rather than fine grained ("pepper-and-salt").

Demerec and Slizynska (1937) reported the first observation of the spreading effect. In $T(1;4)w^{m258-18}$, the distal tip of the X, to 3C3, is translocated to 101F. The areas of the eye with the rough phenotype, associated with deficiency or mutation in 3C3–6, were larger than, and completely included, all areas that were white. Although consistent with the hypothesis that the euchromatic region rendered inactive in a cell has uninterrupted contiguity with the breakpoint, though the extent of this inactivation differs from cell to cell, this single instance is subject to alternative explanations. For example, the inactivated chromosome regions may indeed always be contiguous but, if so, they may always be of the same extent—inactivation of the 3C5–6 region by the distally placed heterochromatin of 101F might instead have been invoked to explain the pigmented but roughest regions. Or, alternatively, loci in 3C3 may be inherently more susceptible to inactivation than *w* in 3C2. Other rearrangements with other breakpoints, or other modes of observation of the variegation phenomenon were required to settle the point. For this particular translocation, Schultz (1939) noted that the translocated X tip was well banded in the salivary gland chromosomes to a point in region 3C which varied from cell to cell; the entire part nearest the breakpoint was often not visible at all ("heterochromatized") and presumably not polytenized.

For a somewhat different translocation, ($T(1;4)w^{m258-21}$) with X break at 3E5/6, Hartmann-Goldstein (1967) published a very extensive study of the extent of "heterochromatization" in the salivary gland cells of flies

subjected to a variety of conditions. Band "3C1" was clearly visible (polytenized) in more cells than was 3C7, and was always visible when 3C7 was visible. The cytological "variegation" correlated well with the extent of white and Notch-variegation (see Section III, B).

When variegation is induced at both ends of an insertion, as in *Dp(1;3)*N^{264-68}, w^+ and fa^+ can be inactivated separately: all four possible combinations (w^+ fa^+, w^+ *fa*, *w* fa^+ and *w fa*) of phenotypes can be found in patches of the eye (Cohen, 1962). Panshin (1938) found extreme white variegation—with w^+ suppressed in nearly all the eye—to result from the replacement of the distal part of the X tip in *T(1;4)*w^{m11} (in which 3C2 is already next to 4L) by 3R, bringing nearly the whole of the heterochromatic base of that arm to the distal side of 3C2. It is very likely that the more frequent inactivation of w^+ when thus sandwiched can be interpreted as due part of the time to spread from its neighbor on the left (3R) and the rest of the time to spread from its neighbor on the right (4L). If so, this is consistent with Cohen's observations on the insertion into 3L, and implies that separate heterochromatic regions act as separate origins of inactivation not rigorously synchronized, of the "euchromatin" lying immediately adjacent. Loci nearest the boundaries of the insertion are inactivated most frequently and may even be interpreted as "stable mutants" as had been the case for *dm* in 3D4–5 in *Dp(1;3)*N^{264-68}. Vital loci near that boundary are sufficiently inactive that this insertion completely failed to cover at least 6 deficiencies in that region that were covered well or poorly by a non-variegating insertion, with the same proximal boundary, into the midst of 2R euchromatin (Ratty, 1954). The left end of this insertion can to some extent cover 6 of the *z-w* essential loci (Kaufman, 1970) although bands corresponding to the two left-most are never seen in salivary gland preparations.

An interesting but as yet barely examined question concerns whether a comparable spreading effect exists for the variegation of heterochromatic loci. It is likely that the basis of their variegation is distinct from that of euchromatic loci. It would then be possible that a situation uncongenial to the action of some loci accustomed to a position near or in heterochromatin may not be so uncongenial to the action of others similarly situated.

In the single instance in which it is quite clear that the heterochromatic loci are indeed normally all active at the same time in the same cells, the typical spreading effect is seen. The Y loci for lampbrush loops are active during prophase in the primary spermatocyte of *D. hydei*. In the X–Y translocation mentioned in the previous section, occasionally the loops unfolded. Sometimes all were active, and sometimes only the loops farther from the breakpoint (Hess, 1970a).

III. The Variegated Phenotype

A. Variegation is Localized Gene Inactivity

So far in this discussion I have simply assumed that patches of mutant phenotype contained cells in which the relevant genes were inactive, rather than partially active or coding for abnormal products. The time has come to examine this assumption.

This examination can be conducted critically only in a test system that limits the detectable variegation to a single locus, and thus we use heterozygotes that combine the rearranged chromosome and a normal chromosome bearing a recessive point mutant at a locus exhibiting the usual "recessive" variegation. In homozygous or hemizygous rearrangements, there may be closely linked loci of interrelated function all or only some of which are subject to inactivation via the spreading effect, with complex phenotypic consequences.

Probably the most clear-cut demonstrations of reduction, but not qualitative alteration, of gene product by the locus subject to variegation are given for the enzyme loci *Amy* and *Pgd*. Bahn (1971) electrophoresced heterozygotes for *T(1;2)OR32*, which brings the Amy^{2} allele of the amylase locus near X heterochromatin, and a normal second chromosome bearing the $Amy^{4,6}$ allele, scoring individuals with various sex-chromosome constitutions on the same gel to permit comparisons. The intensity of the stain at the position of the Amy^{2} amylase allozyme was directly related to factors that enhance or suppress variegation, while the intensity of the $Amy^{4,6}$ allozyme remained constant. X0 males lacked detectable activity of the Amy^{2} allozyme. Also, Gerazimova *et al.* (1972) report that conditions that elicit variegation of *sc*, *dor* and *pn* in *Dp(1;f)R*—low temperature in X/Dp/0 males—reduce the measurable specific activity of 6-phosphogluconate dehydrogenase by roughly 25% of its activity in X/Dp/Y males. When the X chromosome carried Pgd^{B}, the activity of the allele Pgd^{A} in the duplication could be distinguished, though not quantitated, on electrophoretic gels. The A and hybrid bands were lighter in X0 than in XY males.

In short, from the point of view of the whole fly, the enzyme loci in these two rearrangements act as hypomorphs, genes of qualitatively identical but quantitatively lesser effect than wild type (Muller, 1932). This had been shown much earlier for "morphological" loci, e.g. the forked locus in an extensive study by Belgovsky (1946). Whole-fly hypomorphism is the consequence, most probably, of the averaged effects of two kinds of cells in which the locus is normally expressed—cells in which the locus is fully active and cells in which it is inactive. This is the simplest

conclusion to be derived from the many observations of variegation as a mosaic of wild-type and amorph mutant phenotypes.

Judd (1955) compared the phenotypes of *T(1;4)*$w^{m258-21}$ containing w^+, w^a or *w* in the translocation when heterozygous with an array of white alleles including w^+, w^a and *w*. In each case, the phenotype was that expected from uniform action of the allele in the normal chromosome and full action in some cells, complete inaction in others, of the allele in the translocation.

In an electron-microscopic study of the anatomy of white variegation in *w/w*; *Dp(1;3)*N^{264-58}, Shoup (1966) found that whole ommatidia resemble either Oregon-R or *w* ommatidia, all pigment cells having the complete granule complement of one or the other. When more than one locus may have been variegating, in *T(1;4)*$w^{m258-18}$, Gersh (1952) found that the two primary cells of a single ommatidium could be of different phenotype—the one fully pigmented and the other white—but that in the mutant regions some of the secondary cells showed a third phenotype, with many pale granules.

There are even instances in which the most extreme available point mutant is not an amorph but a hypomorph, such as *ci*. For example, triplo-4 *ci/ci/ci* are less extreme in phenotype than *ci/ci*. There are some *T*(ci^+) giving more extreme phenotypes, such as *T*(ci^+)/*ci*, than seen in *ci/ci*, although *T*(ci^+)/*ci/ci* is always less extreme than *T*(ci^+)/*ci* (Stern *et al.*, 1946). This is at least partly attributable to complete inactivation of the *ci* locus in the translocation in some cells, although inactivation of adjacent interacting loci may also contribute (see Section II, E).

B. Tissue Differences in Susceptibility to Inactivation

Variegation of a locus can be detected in every organ of the fly in which the locus normally acts. However, the level of variegation characteristically differs from one organ to another so that the locus is very frequently inactive in one, but very infrequently so in another.

Here again the white locus has been the most extensively studied. It is expressed in eye, Malpighian tube, and testis sheath; within the eye it is expressed in the pigment cells of every ommatidium. In addition, its condition in salivary gland chromosomes can be observed. There are regional differences within the eye. For several different rearrangements the posterior region of the eye is more likely to be pigmented than any other, occasionally the entire remainder of the eye being white. The antero-ventral sector is most often white (Gersh, 1952; Becker, 1961). The eye as a whole is the organ in which the highest proportion of cells are white.

The proportion of mutant cells in testis sheath is lower (Hessler, 1961) though it has not been compared to those in the Malpighian tubes (or, of course, in the salivary gland) of the same animal. For *T(1;4)*$w^{m258-21}$, Schultz (1956) reported that when far more than half the area of the eye was mutant, pigment nevertheless occurring in sizeable patches, approximately half of the Malpighian tube cells were white in a fine-grained, each-cell-on-its-own mosaicism. The average percentage of white cells in the Malpighian tubes is virtually the same as the average percentage of salivary gland nuclei in which the 3C2 band was not clearly distinguishable, in larvae (Hartman-Goldstein, 1967). Breughel (1970) noted that for the various white-mottled rearrangements in *D. hydei*, a pepper-and-salt mosaicism in the eye accompanied a high proportion of white cells in the Malpighian tubes while a large-spot mosaicism in the eye accompanied few and scattered white cells in the Malpighian tubes.

Factors which increase w^+-locus inactivity in the eye do so also in the testis sheath (Hessler, 1961) and Malphighian tubes (Schultz, 1956), and lead to "heterochromatization" (probably lack of polytenization) of 3C2 in a higher proportion of salivary gland nuclei (Hartmann-Goldstein, 1967). However, for individuals of a single genotype reared under similar conditions, there is no further correlation between the extent of variegation in these various tissues (Hessler, 1961; Hartmann-Goldstein, 1967) signifying that the inactivation process occurred independently during the development of the separate tissues. Yellow pigment develops earliest in the Malpighian tube, before the larva hatches from the egg. In the eye, the pigments begin to appear half-way through the pupal stage, and appear synchronously throughout the eye even though the ambient temperature is so low (14°C) that the time from puparium formation to eclosion of the adult is extended to 13 days (Gersh, 1952). Pigment (sepiapteridine) appears last in the testis sheath, during the first day after adult eclosion. Thus, no clear correlation exists between the time of onset of pigment formation and the tendency for w^+ inactivation although there may be a relation between the latter and the number of mitoses in the ancestral lineage of the indicated cell.

Another locus, *y*, has been studied for its tendency to variegate in different parts of the body. Noujdin (1936) noted that the probability of a bristle being yellow (due to *In(1)*sc^8) depended on its position in the mesonotum. Gsell (1971) ranked the probability of bristles being yellow (again, due to the same sc^8 heterochromatin–euchromatin junction, but translocated to the tip of Y^L and in a variegation-enhancing genotype) from highest (71%) in the genital arch and claspers; next highest in the four regions: anal plate, antennal segment II, anterior edge of the wing, and sex comb; less in the outer edge of the femur; still less (11%) in

antennal segment III; to rare occurrence in abdominal tergites or sternites. He did not rank any part of the mesonotum.

One last example of variegation in different tissues: Clancy (1964) observed that translocations affixing dor^+ to the Y chromosome variegate for dor^l, expressed as reduced viability of $dor^l/T(1;Y)$. In the survivors, in which dor^+ must have been active in a critical number of cells, the eye pigment is nonetheless strongly variegated.

C. Variegation when Point Mutants have Variable Expression

Often, variegation of a locus is manifested by its quantitative effect on a trait, the cells in which the locus is transcribed being distant in time or place from the region in which the phenotypic effect is displayed. Thus, the v^+ cells of the fat body in which tryptophan pyrrolase is formed are remote from the eye in which ommochromes are formed and the cells in which ci^+ acts are not necessarily those forming the cubitus vein. And, in both cases, variegation has been identified through quantitative (and variable) reduction in the product.

In the case of vermilion, variegation in the strict sense could easily be demonstrated by fluorescence of the kynurenine-producing cells of the fat body (Rizki, 1961). The tryptophan pyrrolase specific activity was found to be about 50% of normal for the *v* locus in $T(1;2)ras^V$ and about 80% of normal in $T(1;Y)y^+Yv^+$ (Tobler *et al.*, 1971), while still subject to the usual dosage compensation mechanisms. Nevertheless, I do not know of any direct attempt to visualize mosaic expression of this locus in these rearrangements in the fat body.

It would also be possible to screen for *mal* variegation of the Malpighian tubes directly by histochemical means. Janning (1972) has shown this to be feasible by correlating the loss of the ring from *R(1)2/y w mal* females with loss of stainability for aldehyde oxidase in individual cells, using w^+ as a marker for the ring. In other tissues in which *w* is not expressed, mosaicism for aldehyde oxidase was still evident.

Another measurable reduction in gene product associated with variegation has been shown for ribosomal RNA in $In(1)sc^{S1}$ and $In(1)sc^{L8}$ (see Section II, C,2). Nix (1973) found that third instar $y\ sc^{S1}/0$ larvae have about 85% as much rRNA per wet weight as their $y\ sc^{S1}/y^+Y$ controls, although he showed the number of rDNA cistrons to be normal. 4S RNA was unaffected. The reduction in ribosomal protein was even more severe. Puckett and Snyder (1973b) have evidence that the $sc^{S1}/0$ embryo synthesizes so much less rRNA during the first five hours of embryogenesis that the newly hatched larva had 14% less rRNA than $In(1)dl\text{-}49 + In(1)B^{M1}/0$

controls, after each was standardized against its female and metafemale siblings. The rate of synthesis after the first five hours was the same. It is possible that transcription was initiated later in most cells of the $sc^{S1}/0$ embryo, so that the difference is ascribable solely to different durations of transcription. In this case, descendants of cells that transcribed rRNA normally in the embryo subsequently cease transcription in $sc^{S1}/0$ larvae, so that the 14% difference persisting in the newly hatched larva is not virtually obliterated by late third instar. It is also possible that the population of cells particularly active in transcribing rRNA during the latter three-quarters of embryogenesis is one in which the rDNA cistrons are not inactivated in the sc^{S1} chromosome. Other cell types, normally particularly active in rRNA transcription in the earliest hours after blastoderm formation, less active in the latter part of embryogenesis, and reactivated during larval development, may be those more susceptible to nucleolar-inactivation in the sc^{S1} chromosome.

Meanwhile, the eggs produced by sc^{S1} or sc^{L8} mothers have a relative deficit of rRNA at the time of fertilization. The normal oocyte eventually has roughly $1{\cdot}9 \times 10^{10}$ ribosomes, synthesized for the most part by the 15 polytene nurse cells (Klug *et al.*, 1970). The supply is sufficient to sustain development through most of embryogenesis, since *y* sc^{4L} $sc^{8R}/0$, totally lacking in rDNA cistrons, die as early first instar larvae or late embryos [distinguishable from their nucleolus-organizer-bearing sibs by yellow mouthparts (Barr and Markowitz, 1970)].

Puckett and Snyder (1973a) compared the rate of synthesis of rRNA in ovaries of females homozygous and heterozygous for sc^{S1} or sc^{L8}. The rate of synthesis was the same in whole ovaries, but the total amount synthesized in homozygotes was less than 90% of the amount synthesized in heterozygotes. Unfortunately, the other chromosome in the heterozygote in this study was FM6, which is derived from $In(1)sc^{8}$, suggested by Baker to have a less severe position-effect on the nucleolus organizer. The difference between homozygous and heterozygous sc^{S1} ovaries could well have been greater if a clearly non-variegating balancer chromosome had been used.

However, in the numerous cases of loci whose mutants themselves have quantitatively variable effects, and whose RNA or protein products have not been identified, the possibilities for distinguishing between position-effect and point mutation (near or at the breakpoint of a rearrangement) are limited and not often decisive. Classic cases are offered by *h*, *ci* (the "Dubinin effect") and *sc*. For all of these, the point mutant itself has a variable expression. At least one $T(3;4)h^{V}$ with "weakened dominance" (i.e. when heterozygous with *h* it showed a slight increase in the number of hairs in several of the sites affected by the locus) was employed in the crossover demonstration that the allele in the translocation was the wild

type h^+, whose normal function was blocked because of proximity to a heterochromatic breakpoint (Section II, A.1). Yet the variability of the position-effect phenotype is often no greater than that of the point mutant, while its average expression may be even more extreme. The decision that a given case results from position-effect variegation must then often rest, rather insecurely, on the nature of the rearrangement putatively generating it and on the modifiability of expression by factors commonly affecting known cases of position-effect variegation (see Section IV). And these studies, since expression is so variable, usually involve comparison of siblings some of whom have the rearrangement genotype and others, a "standard" homozygous genotype.

D. Homozygotes, Hemizygotes and Heterozygotes

In any large-scale attempt to induce variegation for a particular locus, more than half of the rearrangements so induced are lethal as homozygotes (or hemizygotes). This is easily understood as extreme variegation for essential loci that lie nearer the breakpoint than the locus under study.

For those rearrangements that can be obtained as homozygotes, the phenotype of the homozygote can be compared with that of the heterozygote with the amorph allele on the normal chromosome. When hemizygotes can be obtained, the phenotype corresponds more closely to that of the homozygote. In the majority of cases, the homozygous rearrangement gives the more nearly wild-type phenotype. The obvious interpretation is that the inactivation of the locus is a separate event in each homologue and the presence of one active locus guarantees the dominant phenotype. Among examples of this uncomplicated homozygous expression can be cited the large-spot white variegations (Hessler, 1961; Spofford, unpublished observations; Breughel, 1970 for *D. hydei*), hairy (Dubinin and Sidorov, 1935; Jeffery, 1972), roughest (Kaufmann, 1942), and most instances of brown variegation (Slatis, 1955a).

Fine mosaicism ("pepper-and-salt") of the white locus tends, on the other hand, to a more strongly mutant expression in the homozygote, both in *D. melanogaster* (Schultz, 1939) and in *D. hydei* (Breughel, 1970). In the small number of cases at hand so far in *D. hydei*, fine-grained mosaicism is associated with breaks at some distance away to the right of the white locus while large spots are associated with a break to the left. Breughel suggests the difference in homozygous expression may result from a regulatory locus for white lying to the right of the structural locus. The regulatory locus in the normal-sequence chromosome would be active even though the structural locus was mutant so that in the heterozygote

the inactivation would have to spread as far as the structural (*w*) locus for a white spot. In the homozygote, the inactivation need spread only as far as the regulatory locus in both chromosomes.

A different explanation from the uncomplicated one offered earlier for a more nearly wild-type phenotype in homozygote than heterozygote for a rearrangement is necessary for *ci*. Here, the structural heterozygote is more extreme in phenotype than the hypomorphic *ci*/*ci* (Dubinin and Sidorov, 1934; Stern *et al.*, 1946; Stern and Kodani, 1955). The near-lethality of homozygous and hemizygous rearrangements, when they survive at all, suggests that an essential locus lying near the breakpoint is also variegating and that only cells with this locus active (and, thus, ci^+ also active) survive during development. The *ci* locus, however, introduces yet another phenomenon that must be discussed next.

E. "Dominant" Variegation

There are at least three cases, two of them well documented, in which, apparently, the variegation is dominant, e.g. $g\ bp/g^+\ bp^+$ in Baker's terminology (or $R(g)/g^+$ in Stern's) shows mutant expression when the mutant *g* is itself normally recessive to g^+. These three cases concern the *ci* and *bw* loci and the eye phenotype associated with inactivation of at least two otherwise unidentified loci, one at each side of the euchromatic breakpoint of *In*(*2LR*)*40d* (Hinton, 1949). Enough is known of the first two instances to attempt an explanation that brings these in line with the far more usual situation discussed already. Direct attempts to induce dominant variegation at other loci—in particular *w* (Burkholder, 1954, and personal communication) and *h* (Jeffery, 1972)—have had a predictable lack of success, these being the very loci used in the crossover demonstration that their variegation was a position effect (Section II, A,1).

1. *Cubitus Interruptus*

Hochman (1971) places the *ci* locus somewhere in 101F–102A1–2 since it is uncovered by the deficiency $M(4)^{63a}$. The heterozygote $M(4)^{63a}/ci^D$ is viable and has a phenotype like $+/ci^D$, less extremely mutant than ci/ci^D. There is thus a distinct locus at which mutation (probably hypomorphic or amorphic, since the ci^D lethality involves at least two complementing essential loci) dominantly gives rise to a *ci*-like phenotype. For purposes of discussion, I will call it $Su(ci^+)$. One of many speculations on the function of the type allele at this second locus is that its product is necessary for normal function of the *ci* locus in both cis and trans position, and is

present in limiting amounts so that less ci^+ or *ci* activity results when less $Su(ci^+)^+$ product is made.

Typical recessive variegation of ci^+ requires a break to the left of 101F. However, a less striking but no less real variegation resulting from a translocation induced in a *ci* chromosome can also involve breaks just to the left of 102B1 (Stern and Kodani, 1955). This variegation can be expressed whether the normal sequence chromosome carries ci^+ or the hypomorph *ci*. Stern and Kodani (1955) also found three variegating $R(ci^+)$'s with breaks just to the left of 102B1, but did not report the phenotype of these in $R(ci)^+/ci^+$. Breaks immediately to the right of 102B1 sometimes gave a weak *R(ci)* variegation when in combination with *ci*.

It seems to me likely that it is the $Su(ci^+)$ locus whose variegation is responsible for the effects in $R(ci)/ci^+$ genotypes. I would suggest 102B1 as the likely position for this locus. In *R(ci)/ci* inactivation of either the hypomorph *ci* or $Su(ci^+)^+$ would contribute to the phenotype.

2. *Brown*

Almost half of the rearrangements induced in bw^+ chromosomes that variegate for brown do so even when heterozygous with a bw^+ chromosome (Slatis, 1955a). The rate at which dominantly variegating rearrangements can be induced is the same whether the brown locus is occupied by the wild-type or the amorph allele. Slatis noted that the dominance of the variegation increased with the distance of the break from the probable locus of brown, to a maximum at about 6 bands away on either side. The phenotypes of most of the possible homozygotes and heterozygotes for hypomorphic or amorphic alleles or for two variegating rearrangements could be reconstructed by postulating for each rearrangement a particular value for the dominant effects (bw^+ inactivation regardless of cis or trans relation to the breakpoint) and a separate additive value for the "recessive" effects (limited to the cis bw^+). This lends credence to the idea that two distinguishable loci, both affecting *bw* locus function (one being *bw*) are subject to inactivation in these rearrangements. In fact, since there are two regions of maximal dominant effect, the second locus affected in some instances lies to the right of *bw* and in others, to the left. Presumably both "second" loci would rarely be affected by a single break.

A candidate for one of these *bw*-regulating loci is listed by Lindsley and Grell (1968) as $Su(bw^{V1})$. The mutant, discovered by Kadel (1959), has many of the recombinational and reversional properties of a duplication, and is placed at 2–105·2, 0·7 units or, very approximately, 10 bands to the right of *bw*. For the sake of constructing a plausible hypothesis, we can assume the mutant discovered to be a hypermorph if indeed it is a duplica-

tion. Its effect is to reduce dominant brown variegation. The unduplicated normal allele could be proposed to facilitate the functioning of—i.e. regulate—the bw^+ allele. Its inactivation in a rearrangement would then hamper bw^+ functioning in either cis or trans position.

Thus, similar explanations can be invented for both *ci* and *bw* dominant variegation. A closer clustering of the relevant structural and regulatory loci, such as probably exists in the complex bithorax system, would have been nearly unresolvable while no confusion would have arisen at all if they were too distant for the effects of single breakpoints to spread from one to the other.

F. Clonal Expression and the Timing of the Variegation Event

For some rearrangements, the mosaicism is so fine-grained that the final decision whether the relevant locus is to be active or not must have been deferred until late in development—for example, until early pupa for $T(1;4)w^{m258-18}$. The appearance of localized pigment in various positions along the axis of the scutellar bristles in $In(1)y^{3P}$ males (Spofford, unpublished observations) suggests that the decision is not irrevocable and may occur within a single polytene cell as late as the first day of pupation.

Nevertheless, for other rearrangements the inactivation occurs very early in development, certainly during the first five hours of embryonic development for the rDNA cistrons in $In(1)sc^{S1}$ (Section III, C). In still other cases, the patches within which loci are inactive are the clonal descendants of cells within which the decisions were largely, if not irrevocably, made. The size and location of these clones indicate the decision to be very early.

Noujdin (1936) attempted to interpret pattern in terms of cell lineage in the mesonotum and relate it to the time of decision of *y ac* variegation in $In(1)sc^8$. He found nine semi-independent regions on each side within which mutant expression in individual bristles is far more highly correlated than it is between regions. The boundaries between these regions could not, however, be drawn absolutely sharply, as if there were a certain degree of plasticity in the development of the mesonotum. The half-scutellum constituted one region, and a stripe bordering the midline another. Spots in the humerus were completely independent of any part of the mesonotum, as would be expected given its derivation from a separate imaginal disk. He did not use an independent method, such as crossover-generated twin spots, to establish cell lineages. However, Bryant (1970) and Garcia-Bellido and Merriam (1971) have presented data from which it is possible to estimate the number of progenitor cells of the mesonotum

at various larval ages. Bryant obtained five spots and the latter investigators, two spots, each spot representing the descendants of one of the daughters of an irradiated cell in the newly hatched larva. These spots varied considerably in size, depending on position, but suggest the presence of from 3 to 10 mesonotum progenitor cells in the newly hatched larva. The average spot size was halved when crossovers were induced at the end of the first larval instar, signifying the occurrence of at least one round of mitoses during the first instar. The usual clone shape is rather square, but the boundaries are irregular and vary from fly to fly. The rough correspondence between the number of semi-independently variegating regions and the estimated number of progenitor cells indicates that the decision for later activity of y^+ and ac^+ in the sc^8 tip had been made by the time the larvae hatched and could have been made at the time of blastoderm formation. The cells of the wing disc anlage are probably derived, without division, from blastoderm cells. Various estimates have been given for the number of blastoderm nuclei committed to mesonotum formation, based on the XX/X0 boundaries in gynandromorphs (11 nuclei—Garcia-Bellido and Merriam, 1969, or 17—Ripoll, 1972) or crossover spots induced in early embryos (2 or 3 nuclei—Bryant, 1970). Since irradiation of early stages delays development and probably causes cell death, the higher estimates probably come nearer the mark for normal development.

On the other hand, the decision even for this same euchromatin-heterochromatin junction can be much later. In a much more recent study of sc^8 Y-induced *y-ac* variegation, Gsell (1971) reported that the average size of mutant spots was similar to that Bryant and Schneiderman (1969) generated by inducing crossing over in the latter part of the second larval instar, but that the variegational spot sizes were quite variable.

The time of decision is no later than the end of the first larval instar for the large-spot eye pigment patterns for *w* in $Dp(1;3)N^{264-58}$ in *D. melanogaster* and *pe* in $T(Y;5)pe^{m1}$ in *D. virilis* (Baker, 1967). The boundaries between mutant and non-mutant variegational spots in the ventral half of the eye follow the outlines of clonal twin spots resulting from somatic crossing over induced at that time (Becker, 1957 and 1961; Baker, 1967), when there are eight progenitor cells. Twin spots induced at the same age in the dorsal half of the eye are irregular in location and contour and often not contiguous, as if cell migration has not yet ceased. Even in the pepper-and-salt mottled $T(1;4)w^{m258-18}$, the overall probability that the white locus will be inactivated in individual cells later is determined by this time, whole clones having consistent levels of white-spotting—anteroventral clones having the highest and the tiny posterior clone the lowest (Becker, 1961).

The same distribution of probabilities of mutant expression accompanies

In(2LR) 40d (Hinton, 1949) and *In(1)rst*^{3} (Spofford, 1969). The distribution of proportion of *rst* areas in the two eyes of flies under various conditions is consistent with a decision as to subsequent rst^{+} activity occurring when there were as few as 3 to 6 progenitor eye cells, or as early as the time of blastoderm formation.

Since there is evidence of migration of progenitor cells of the ventral half of the eye also during the first larval instar (Becker, 1957), the correspondence between spot contours in variegation and in twin spots induced at the end of the first larval instar merely sets an upper limit to the age of decision for w^{m}. Both Baker (1967) and Janning (1970) have attempted a more precise synchronization of the two events by inducing twin-spots in a variegation genotype. The boundaries of the homozygous spots were more easily distinguished in the system employed by Janning—w^{a} lz^{50e}/w^{a} *rb* rux^{2}; $Dp(1;3)N^{264-58}$ with Y^{S} or Y^{L} attached in some instances to one or the other X chromosome. Since six phenotypes were distinguishable (red glassy or ruby rough on a red smooth background where w^{+} was active and apricot glassy or white rough on an apricot smooth background where w^{+} was inactive), it was possible to ascertain whether the variegational spots lay within the marked clones or vice versa. There was more variation in the relation of the two kinds of spots at the various ages of crossover induction than would permit a clear conclusion. Nevertheless, the proportion of homozygous spots (and also of twins) that contained both w^{+} and w^{a} tissue declined with advancing age of irradiation, with few mixed spots after the first larval molt. The spotting pattern was the same in eye disks explanted from early first instars and from early second instars cultured in adult female hosts for the usual larval developmental time and allowed to metamorphose in late larval hosts (Janning, 1971). The decision-making process had been programmed well enough into the eye disk so that its usual results occurred in a radically altered environment.

Just how much earlier than the end of the first larval instar the decision is actually made depends on the degree of reversibility pertaining to it. That the early decision is absolutely irrevocable is contradicted by the modifiability of expression by alterations in temperature later, even during pupation. When the variegated phenotype consists of small spots of pigment on a nearly colorless ground, as is the case in "light" lines of $T(1;4)w^{m258-18}$ (Demerec and Slizynska, 1937) or of $Dp(1;3)N^{264-58}$ (author's observation), it is difficult to resist the idea that a few subclones regain the capacity to form pigment granules in an otherwise inactivated larger clone. If the decision to be inactive can be revoked in some descendant cell lines, then studies such as those of Becker, Baker and Janning on the correspondence of variegation and crossover spot patterns

merely indicate the latest time at which the decision, tentatively made at first, is customarily confirmed, subject to later revision only as environmental circumstances dictate. I know of no evidence incompatible with the early tentative decision occurring at the time of blastoderm formation, when the supply of nuclei for each of the imaginal disks (García-Bellido and Merriam, 1969) is adequate to account for the amount of variation in total proportions of mutant and non-mutant tissue in the adult phenotype.

Schultz has repeatedly called attention (e.g. in Hadorn *et al.*, 1970) to the fact that mutant areas are larger in those organs and in those parts of organs that undergo the larger number of mitoses in development, as if the time of decision was much the same for all organs.

The one study in which the reversibility or irreversibility of the inactivation process might hope to have been settled is unfortunately equivocal. Gsell (in Hadorn *et al.*, 1970; Gsell, 1971) has conducted an extensive series of cultures and subcultures in adult females of individual explants from various imaginal disks derived originally from two stocks in which the *y* variegation of the sc^8 tip was genetically enhanced, in one stock on the sc^8 *Y* and in the other on a compound *X·Y*. He presented six transfer culture pedigrees, four with over 200 test implants each. The percentage of yellow tissue in the implants was a characteristic of the subline within a pedigree, regardless of the types of transdetermination of tissue types displayed by an implant. Occasional "mutations" occurred in the characteristic percentage of yellow in some sublines, the new percentage characterizing the derivative subline. 28 sublines became completely wild-type, with no yellow spots recoverable in later implants. No subline became completely yellow for more than one transfer generation—after one generation, these lines died. The variegating condition continued apparently indefinitely in other sublines. After 22 transfer generations, one implant was cut in checkerboard fashion, half the pieces transferred once again and the other half tested. All the pieces tested still developed mosaic phenotypes and after the pieces transferred were also cut into checkerboards and tested at the next opportunity, they too developed mosaic phenotypes. Gsell interpreted this persistent fine-grained mosaicism as the continual sorting-out, not completed even after 24 subdivisions of tissue, of cell lines irrevocably determined before the original explantation. It is much easier to conceive of cell lines with metastable states of inactivation of the y^+ locus in which the final decision occurs stochastically as the tissues approach the pupal metamorphosis, with loss of inactivation the only state stably heritable through repeated transfer generations.

It may be of value here to digress to position-effect variegation in the mouse. The c^+-locus is included in an insertion of a segment of autosome into the X chromosome. The insertion is carried as a duplication in *Dp*; *c/c*

mice. These mice occasionally display colored spots of half-clone size and, in clones with the insertion-bearing X inactive, pigmented hairs appear increasingly as the mouse ages (Cattanach *et al.*, 1969) although white hairs do not correspondingly increase in number in the pigmented clones. When the normal autosomes carry both c^{ch} and p, the first pigmented hairs to develop in a previously all-white clone are $c^{ch}\ p^+$, the p locus being the farther from a breakpoint. Later, $c^+\ p^+$ hairs appear (Cattanach, 1974).

In flies, however, the mosaic phenotypes studied are not expressed until after the final cell divisions; descendants of known single cells have not been sampled sequentially during the history of a cell lineage, and the degree of reversibility of the inactive state and the consequent identification of the time of inactivation remain moot.

IV. Factors Modifying Intensity of Variegation

A major frustration in the study of position-effect variegation is the notorious sensitivity of the phenotype to a variety of factors extrinsic to the rearrangement itself. Elaborate controls are usually necessary to assure that these (especially temperature, background genotype, and even specific details of parentage) are not confounded with the variable under direct study.

A. Temperature

1. Higher Temperatures Usually Suppress Variegation

Temperature was one of the first modifying factors to be reported in the literature. Gowen and Gay (1934) reported that w^{m1}, w^{m2}, and w^{m3} all displayed larger areas of mutant tissue (*w, N, spl, ec*) at lower temperatures than at higher. The typical finding, in most cases, is enhancement of variegation at lower temperatures and suppression at higher. These cases include *w* in many rearrangements, *rst* in $In(1)rst^3$ (Kaufmann, 1942), *m* in $In(1)m^K$ (Wargent, 1971), *pdf* in $In(1)B^{M1}$ (Schalet, 1969), Rev^B in $In(2LR)rev^B$ (Wargent, 1972), *ci* in numerous rearrangements (Stern and Kodani, 1955), and the male-fertility factors in several $T(Y;4)$'s (Benner, 1970). Lethals near *w* (Gowen and Gay, 1934), near *v* and near *ras* (Barr, personal communication) kill a higher fraction of rearrangement-bearing X0 males at lower temperatures. The activity of the enzymes coded by the *Amy* (Bahn, 1971) and *Pgd* loci (Gvozdev *et al.*, 1973) in variegating rearrangements is lower at lower temperatures. The inference one would naturally draw from the usual temperature effect is that a shorter region is subject to inactivation at higher temperatures.

However, there are a number of reasons for not expecting a rule such as that enunciated at the heading of this subsection to be universally applicable. Many mutants themselves have temperature-dependent phenotypes. Some are expressed only at higher temperatures and are usually ascribed to a greater heat-lability of the protein coded by the mutant allele. Some are expressed especially at lower temperatures and are usually ascribed to altered kinetics of reactions affected by the mutant or defective enzyme. For example, *lt* eyes are lighter at higher temperatures. Gersh (1949) could find no effect of temperature on the lt^m phenotype in $T(1;2)w^{258-39}$. On the other hand, *ci* expression is more extreme at lower temperatures, so that Stern and Kodani (1955) are reluctant to ascribe the more extreme expression of $R(ci^+)/ci$ at lower temperatures to an effect on the variegation process itself. The scutellar bristle number in $In(1)sc^4$ is related inversely to temperature (Mampell, 1965b), and seems to depend on the duration of the developmental period.

Furthermore, neighboring loci near a breakpoint may individually affect the same ultimate phenotype but in opposite ways. In this case the phenotype would not change monotonically with increasing temperature.

There are several instances of non-linear temperature effects. The eye phenotype associated with *In(2LR)40d* is most extreme in the middle range of temperatures (Hinton, 1949). Some of the effects of $In(1)sc^8$ are more extreme at extreme temperatures than near 25°C (Gersh, 1949), although since Prokofyeva-Belgovskaya (1947) finds this to be true also of the cytological appearance of the tip of this chromosome in the salivary gland, the interpretation may be more difficult than I have suggested.

Processes which occur in mutant individuals more normally at higher than at lower temperatures cannot usually be referred to the heat-lability of individual proteins. If the variegation process in any way depends on the properties of temperature-senstitive proteins, these proteins would most likely have roles in the inactivation process. One, perhaps relevant, type of process that is sensitive to cold, for which mutants may be especially sensitive, is the self-assembly of protein molecules into aggregates of specific three-dimensional form. Frankel (1973) reports a mutant Y fertility factor whose sensitivity to cold occurs at the time of organelle formation during spermatid maturation. So, an examination of the temperature sensitivity of particular variegation systems at different stages of development is in order.

2. *The Temperature Sensitive Period*

The stages of development in which temperature is critical to the variegated phenotype have been examined for few systems other than those giving

white mottling: *T(1;4)*w^{m5} (Chen, 1948), *T(1;4)*$w^{m258-18}$ (Chen, 1948; Becker, 1961), *T(1;4)*$w^{m258-21}$ (Schultz, 1956; Hartmann-Goldstein, 1967), and *Dp(1;3)*N^{264-58} (Janning, 1970a; Spofford, unpublished observations). Chen, Becker, and Janning agree in finding a major period of temperature-sensitivity during the first two days after puparium-formation. The most extensive data were given by Chen, using larvae all of whom had eclosed from eggs laid and kept at 25°C. When part of the temperature-sensitive period was passed at 25°C and the remainder at 16–17°C, regardless of temporal order, the net effect on the phenotype could be predicted from the relative duration of time at each temperature (giving greater weight to the second day of pupation for $w^{m258-18}$ but weighting the two days equally for w^{m5}). The period of sensitivity began earlier and continued throughout pupation when the second or third chromosomes of the stocks were replaced by *bw* or *st*, respectively.

Schultz, Hartmann-Goldstein, and I have also investigated the possible temperature-sensitivity of embryonic stages, and find the first few hours after egg laying to be as important as the pupal stage. In the most extensive of these studies, Hartmann-Goldstein assayed white variegation in Malpighian tubes, variegation of bands 3C1 and 3C7 in salivary gland chromosomes in late third-instar larvae, and Notch variegation in adult females. The temperature-sensitive period for the Malpighian tubes was completed by 4 hours, although their first visible differentiation occurs two hours later. The peak temperature sensitivity of polytenization in the salivary glands occurs within the first 3 hours of embryogenesis, the sensitivity abruptly dropping by 5 hours of age and ceasing before the last third of embryogenesis. The Notch phenotype was subject to a peak of sensitivity within the first three hours, although temperature sensitivity continued into the larval period, ceasing before puparium formation. The three hours of embryonic life had the same importance as the entire remainder of developmental time in the effect on the Notch phenotype.

I examined white variegation in adult eyes in two stocks, one homozygous for an extreme enhancing allele and the other homozygous for an extreme suppressing allele at the *Su(var)* locus (see Section IV, G). Both stocks showed an early onset of temperature sensitivity, at around the time of blastoderm formation. In the variegation-enhanced stock, temperature sensitivity continued throughout larval development, both types of shift (up or down) being effective and yielding an intermediate degree of variegation depending on the proportion of development undergone at each temperature. In the variegation-suppressed stock, the difference in phenotype between temperatures was less and did not permit so critical a delimitation of the temperature-sensitive period. If the first four hours of embryogenesis occurred at 25°C, the temperature for the remainder of

development was irrelevant to the phenotype, which was almost completely wild-type. If the first sixth of embryonic development occurred at 19°C, a later period of sensitivity in mid-third-instar could be demonstrated. These results can be interpreted as indicating that in variegation, some component of the inactivation process is somewhat thermolabile, this component being much more thermolabile in the variegation-suppressor stock than in the enhancer stock.

Benner (1970) found the variegating male-fertility factors in several *T(Y;4)*'s to be less active in primary spermatocytes subjected to lower temperatures 10 days before the ejaculation of the mature sperm. The sensitive period is thus around the time of the final mitosis or perhaps very early in meiotic prophase, during the initial elaboration of the lampbrush loops and Y-specific RNA transcription. Transcription probably continues, however, for several days, if the process in *D. melanogaster* is similar to that in *D. hydei*.

Thus, it seems to be a general rule that the temperature sensitivity precedes the time at which the affected locus would normally be expected to be transcribed, in some instances by a very long interval. The sensitive period itself can be of long duration, or divided into two separate periods of peak sensitivity. These temporal relations must be accommodated by any hypothesis formulated to account for the variegation process.

B. The Y Chromosome

1. Direct Effect of the Y Chromosome

Just as there is a "typical" phenotypic response to temperature, so there is a "typical" response to the Y chromosome, also reported first by Gowen and Gay (1934) for w^{m1} and w^{m2}. In the vast majority of cases, additional Y chromosomes in an individual's genotype suppress variegation, while fewer than the normal number enhance it. The exceptions are fewer in the case of the Y chromosome than in the case of temperature, possibly since the Y chromosome affects the direct expression of far fewer genes confoundingly than does temperature. Both enzyme loci studied whose variegating allele product was distinguishable electrophoretically, *Amy* in *T(1;2)OR32* (Bahn, 1971) and *Pgd* in *Dp(1;f)R* (Gerazimova *et al.*, 1972), showed graded increases of activity of the affected allele as the number of Y chromosomes increased within a sex. For example, for *Amy*, X0 < XY < XYY and XX < XXY.

Transcription from the variegating fragment *Dp(1;f)R* is lower and more variable in salivary gland cells of XO than of XY males at the same temperature (Ananiev and Gvozdev, 1974).

Variegation of some loci in some rearrangements is so extreme that the phenotype appears uniformly mutant except in the presence of an added Y; the other extreme condition holds in other cases, the phenotype appearing wild-type in XX or XY and variegated only in X0. The first extreme type is exemplified by variegation of essential loci in $T(1;3)N^{264-6}$, $T(1;2)N^{264-9}$ and $T(1;2)N^{264-10}$, all of which can survive only in XYY males (Schultz, cited in Lindsley and Grell, 1968). The second extreme type is exemplified by the variegation of the white locus in $In(1)rst^{3}$ only in XO males (Gersh, 1963). The variegation of essential loci in numerous positions in the euchromatin genome has been shown to be Y-suppressed—near *v* and *ras* (Barr, personal communication), between *z* and *w* (Kaufman, 1970). Lindsley *et al.* (1960) found that roughly one-fifth of the bb^{+} sex-linked mutants they recovered as lethal in X0 males were suppressed to varying extents by the Y chromosome, a result confirmed by Ben Zeev and Falk (1966). Each Y-suppressed lethal was a rearrangement with at least one break at some position on the X chromosome and another at the base of a chromosome arm. One pericentric X inversion required two Y chromosomes for survival. It seems to me likely that the number of position-effect variegations appearing lethal in XY males but not in XYY may be larger than indicated in this study. This is at least one interpretation of Kerschner's (1949) finding that half as many lethal and visible mutations were identified in the progeny of X-rayed XYY males as of X-rayed XY males.

It was once suspected that there would be a difference between the typical variegation systems and those involving heterochromatic or quasi-heterochromatic loci in their response to the Y chromosome. Although there are exceptions to the usual response, they do not all involve heterochromatic loci nor do all rearrangements affecting heterochromatic loci constitute exceptions. The Y chromosome suppresses both the position effects on ci^{+} tested (R. F. Grell, 1959) in XXYY, XXY and XX females and most of the position effect on $Su(ci^{+})$ in comparisons of X0 with XY (Altorfer, 1967), though weak position effects of the latter were sometimes unaffected or even enhanced. Baker (1971) found variegation of the nucleolus organizer in sc^{8}, sc^{L8} and sc^{S1} to be suppressed by the Y chromosome. He had earlier found that an additional Y slightly suppressed the variegation of *pe* in *D. virilis* in $T(Y;5)pe^{m1}$ (also Schneider, 1962). Among the exceptions are rearrangements affecting euchromatic, heterochromatic, and quasi-heterochromatic loci. One euchromatic locus with an exceptional response may be *sc*. Mampell (1965a, b) reported evidence strongly suggesting that the Y chromosome enhances rather than suppresses the scutellar bristle phenotype of $In(1)sc^{4}$. I have repeated his experiments with stricter controls for other possible modifiers of variegation and find a

slightly greater reduction in the number of bristles on the vertex of the head in sc^4/Y than in $sc^4/0$ sons of *C(1)RM*, *y w* mothers, though not on the side of the mesonotum or on the scutellum. In both Mampell's and my experiments, the possibility remains that the effect is attributable to the maternal Y chromosome constitution (see Section IV, B, 4).

The Y chromosome enhanced the mutant effect of one of the rearrangements evoking "dominant" *ci* variegation, with a break separating the presumptive *ci* and $Su(ci^+)$ loci (Altorfer, 1967).

Exceptions among the heterochromatic loci include *lt* and the male fertility factors. The number of Y chromosomes is directly correlated with the extent of mutant tissue for all *lt*-variegating rearrangements tested (Schultz, 1936; Baker and Rein, 1962). For the *lt* locus, the Y chromosome acts as a variegation enhancer. On the other hand, for the male fertility factors in variegating *T(1; Y)*'s in *D. hydei* at least, extra Y chromosomes are without effect on the percentage of males in whom the affected lampbrush loops unfold (Hess, 1970a). The extra Y introduced was a *T(Y; A)* with functional but complementary fertility factors.

2. *Localization of the Variegation-modifying Regions of the Y*

The availability of fragments of the Y chromosome, both free and attached to other chromosomes, permits some degree of localization of the sites on the Y whose hyper- or hypoploidy in the genotype particularly affects variegation. The available reference points are the nucleolus organizer, the size of each arm at metaphase, the ability to complement tester-Y's deficient for specific male fertility factors, and, in *D. hydei*, the location of the specific types of lampbrush loops. Since many of the available Y fragments include unknown portions of potentially effective basal X heterochromatin as well, a certain degree of caution has necessarily been introduced into the interpretation of some of the results.

The initial suggestion that the variegation-suppressing property was not homogeneously distributed along the entire extent of the Y chromosome came from a comparison of the effects of fourteen different free Y fragments (Baker and Spofford, 1959) on the amount of drosopterin in the eyes of $Dp(1;3)N^{264-58}$ flies. Although the seriation of effectiveness of these Y fragments depended to some extent on the type of X chromosome and sex of the fly, in no series did the effectiveness correlate well with the length of the Y. Of four ring Y fragments of apparently equal length, containing all of the KL factors, the one retaining the nucleolus organizer was much the weakest suppressor. The same Y fragments (even from the same fathers) that most strongly suppressed *w*-variegation in $Dp(1;3)N^{264-58}$

most strongly enhanced *lt* variegation in *T(2;3)lt*^{m100} (Baker and Rein, 1962). Those with little effect on *w*^{m} had little effect on *lt*^{m}. It is likely that the same regions are responsible for both effects.

Using variegation of B^S in certain rearrangements as his assay system, Brosseau (1964) found a major suppression site near *kl-2* and another of nearly equal importance proximal to *ks-1*. Since the nucleolus organizer can be seen in some of the less efficacious Y^{cL} rings (Baker and Spofford, 1959), it is possible that the site in Y^S is distal to the nucleolus organizer. It is noteworthy that in the vicinity of *kl-2* two apparently antithetical properties are localized—the property of suppressing variegation due to rearrangements involving breakpoints elsewhere, and the property of evoking variegation of at least the ci^+ gene when the latter is placed distal to it. This paradox is another item of importance to our understanding of the variegation process.

In *D. hydei* the cytological locations of the male fertility factors are more readily visualized and probably more evenly distributed along the Y chromosome length. Hess (1970) found two major white variegation suppressing regions on the disproportionately longer Y^L of this species, one midway, between the "tubular ribbon" sites and the "pseudonucleolus" site and the other very near the distal tip, present whenever the "thread" site is. In *T(1; Y)*'s with loop sites inactive but present, the suppressor sites remained active.

3. Cell Autonomy of the Y Effect

Gearhart transplanted eye disks from *w*; *Dp(1;3)N*$^{264-58}$ larvae into *C(1)RM*, *y w* larval hosts that possessed or lacked a Y chromosome. The drosopterin content of the implant eyes was the same for both host types and was little different from that of sibs of the implant donors that had been allowed to complete their development normally (Gearhart and MacIntyre, 1971). The effect of the Y chromosome did not pass the disk boundary.

Even within the disk, the effect is cell autonomous. In Janning's (1970) induction of twin spots in *Dp(1;3)N*$^{264-58}$-containing larvae, one of the two X-chromosomes sometimes had a distally-appended Y^S or Y^L, so that one of the resulting twin spots would have two doses of the Y arm and the other, none. The spot with two Y fragments was almost always pigmented, while the spot with none was almost always white. This result also showed that the Y-genotype was of importance to the pigment cell phenotype *after* the end of the first instar when the crossing over had been induced.

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4. Parental Effects of the Y Chromosome

The Y constitution of the parents, particularly of the mother, has proven to be of importance for the extent of variegation in the offspring. This was discovered and first extensively studied by Noujdin (1944) for *y ac* variegation in $In(1)sc^8$ and *y* variegation in $In(1)y^{3P}$ and has been confirmed in several other systems although not in all systems in which it has been checked. A rigorous demonstration of a maternal effect requires that the individuals compared have identical genotypes and the mothers have genotypes identical except for presence of a Y in some but not others. It is not clear to me how well controlled was the autosomal constitution of the flies compared in Noujdin's experiments. However, the sex chromosome constitutions of several matings allow otherwise fairly clear comparisons. E.g. 40·6% of the sc^8/sc^8 daughters of $sc^8/y\ ac$ mothers were mosaic, but only 4·3% of sc^8/sc^8 daughters of $sc^8/y\ ac{\cdot}Y^L$ mothers or 5·3% of $sc^8/y\ ac{\cdot}Y^S$, were mosaic. The suppression of mutant expression is probably attributable to the Y, although other pertinent differences may also distinguish the *y ac*-bearing chromosomes. However, though many other of the comparisons available in his extensive data are consistent with maternal action of the Y chromosome, all other pairs of crosses listed differ also in other features that have been shown to affect variegation in some systems.

Valencia (1947, and personal communication) sought to confirm a maternal Y effect on expression of a series of "dominant" *bw* variegating rearrangements, using a marked Y fragment ($sc\ Y^L$) and carefully establishing co-isogenicity of the background genotypes of the stocks employed. No maternal effect could be demonstrated.

I found a definite maternal effect on the *w*-variegation of $Dp(1;3)N^{264-58}$ of *C(1)RM, y w*/Y daughters when the only difference could have been the mother's Y chromosome. The stocks used were co-isogenic. The crosses were otherwise identical and simultaneous. Daughters whose mothers had had a normal unmarked Y chromosome developed more eye pigment than daughters whose mothers had lacked a Y chromosome. Daughters whose mothers had had various Y fragments with unknown additional contributions of basal X heterochromatin developed the greatest amount of eye pigment. The iso-Y^S was one of the strongest suppressors in these experiments as in Noujdin's (1944). The ranking of a Y chromosome as a maternally-acting variegation suppressor was not the same as its ranking as a direct suppressor. In the sons of these same crosses, the maternal and direct effects were confounded, but predictable on the basis of the maternal effects seen in their sisters and the direct effects ascertained in the daughters of a different series of crosses in which all mothers carried a normal Y and fathers contributed the Y chromosomes to be compared.

Other systems with less elaborate controls have been reported but in which mutant expression appears to be partially suppressed by presence of a Y chromosome in the mother's karyotype. These include the *Amy* variegation system employed by Bahn (1971), w^{m2} in *D. hydei* (Hess, 1970b), and, by an additional Y, $T(1;Y)pe^{m1}$ in *D. virilis* (Schneider, 1962).

A separate effect of the paternal Y chromosome constitution has been reported by Noujdin (1944). When the father's X chromosome (not transmitted to his sons) carried a Y^L or Y^S arm, fewer of his $In(1)y^{3P}$ sons had yellow bristles than if his X chromosome was normal. The paternal effect was relatively smaller than the maternal effects reported. No other worker has reliably confirmed the existence of a paternal effect in any other system.

It would be interesting to discover whether the Y chromosome acts maternally as a suppressor or an enhancer of (or indeed has any maternal effect on) variegation of loci such as *lt* with an anomalous response to the immediate presence of a Y chromosome.

C. The Centromeric Regions of the X Chromosome

If the Y chromosome is usually a variegation-suppressor, one would anticipate that mutant areas would be more extensive in normal females than in normal males, unless some part of the X chromosome were also to act as a suppressor. Greater mutant expression does occur in females in many variegation systems, but certainly not in the vast majority of them.

In certain fairly direct comparisons, a single X chromosome has roughly the same effect as a single Y so that XX and XY have similar phenotypes for the amylase allozyme coded by the Amy^2 allele in *T(1;2)OR32* (Bahn, 1971), or for the eye phenotype associated with *In(2LR)40d* (Hinton, 1949).

Perhaps the most striking demonstration is afforded for brown-variegation by a gynandromorph reported by Grell (1958). The gynander was an offspring of *v*; $In(2LR)bw^{VDe1}/SM1$, *Cy* ♀ × *v*/Y/Y; *bw* ♂. The genitalia were male and the gynander sired offspring. The eye surrounded by female tissue was full vermilion in phenotype, indicating its karyotype to be XXY. The eye surrounded by male tissue was thus most probably XY; it was white with vermilion flecks. The male and female sides were unlikely to have differed by more than a single X chromosome.

What part of the X is most important to variegation-suppression is not disclosed in the studies just cited. Early Russian work suggests that the effective region is in the basal heterochromatin.

Panshin (1938) found that a heterozygous *deficiency* for much of the heterochromatin of the X enhanced the white expression of derivatives of

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$T(1, 4)w^{m11}$. Noujdin (1944) found that $sc^8/sc^8/Dp(1;f)$ females are virtually never mosaic for *y* and *ac* although 6·9% of their *Dp*-free sisters were mosaic. He also found that far fewer (16%) $y^{3P}/Dp(1;f)/Y$ males had patches of yellow bristles than did their *Dp*-free brothers (97%). In addition to its direct effect, this duplication acted maternally. The sc^8/sc^8 daughters of sc^8/sc^8 mothers themselves lacking the *Dp* were twice as likely to be mosaic (13·9%) as when the mothers had the *Dp*. There was a similar effect on sons. A smaller fragment of the centromeric region of the X, including XR, with most of XL replaced by the distal part of 4 carried as a free duplication in the genotype, also suppressed variegation in sc^8/sc^8, comparing mosaicism in sisters with (1·7%) and without (13·03%) this extra element. A possible maternal effect of this duplication was not reported.

In all these instances, the effect of the X chromosome, when present, is in the same direction as the effect of the Y. There is a puzzling situation in which *deficiency* for a part of the X heterochromatin appears to suppress, rather than enhance, a variegation suppressed by the Y. This is the case of certain crossover products retaining the left end of either $In(1)sc^{S1}$ or $In(1)sc^{L8}$ and the right end of $In(1)sc^{V2}$. Although what is known of the heterochromatic breakpoints of these inversions strongly suggests that the crossover product is deficient for a part of the heterochromatin proximal to the nucleolus organizer, the lethality associated with relative inaction of the latter is greatly reduced (Baker, 1971). The mechanism of this suppression may be quite unrelated to the other variegation-modifying action of the X, since here the modifier and the variegating region are parts of the same chromosome, though at opposite ends of it.

D. Centromeric Regions of Autosomes

The bulk of the work on "heterochromatic" modification of variegation has centered on added or subtracted sex chromosomes or fragments thereof. However, at least one autosomal heterochromatic region—at the base of 2R—has had effects similar to the Y suppressors in a limited series of assays. Schultz (in Morgan *et al.*, 1941) reported that $Df(2R)M\text{-}S2^{10}$ is a potent enhancer of variegation. It has been assayed on several systems with usually similar effect, for example on *Bar* in variegating derivatives of the B^SY (Brosseau, 1960). Lindsley *et al.* (1960) found it enhanced 18 of the 23 Y-suppressed lethals they tested.

A new mutant reported by Mange and Sandler (1973), Enhancer-of-daughterless *E(da)*, is a *T(2;3)* with breaks in 66C and in the non-polytenized part of the centromeric region of the second chromosome, probably though not conclusively in 2R. It enhances the mutant *da* (whose effects

are slightly suppressed by the Y) and suppresses the mutant *abo* (also suppressed by the Y), both mutants at loci that have been implicated in the regulation of heterochromatin although not themselves position effects. One might predict that *E(da)* is itself a position-effect resulting from the relocation of a normally heterochromatic locus into distal euchromatin, and that as a phenotypic consequence it mimics a deficiency for the variegation-modifying region at the base of 2R. $In(2LR)Rev^B$, with a breakpoint at the base of 2L, increases the frequency and size of mutant patches due to variegation of the *m* locus at 10E1–2 in $In(1)m^K$, and decreases by 20% the DNA amount (hence, level of polyteny) of the more distant band 10D1–2 in the salivary gland chromosome (Wargent *et al.*, 1974). Duplications for the base of 2R are suppressors (Hannah, 1951; R. F. Grell, 1970). Translocations between the X and an autosome that involve heterochromatic breakpoints in either are often variegation-enhancers (Hannah, 1951; Burkholder, personal communication).

E. Parental Source of Rearrangement

Transmission *per se* through egg rather than through sperm seems to influence the susceptibility of variegating loci in some rearrangements to a later inactivation. These have been called parental source effects. They can be shown convincingly only in reciprocal crosses in which parental sex chromosome constitution and other genetic factors known to affect the final phenotype are held constant while only the rearrangement (preferably autosomal in mode of inheritance) itself is introduced paternally in the one cross and maternally in the other.

The autosomally inherited $Dp(1;3)N^{264-58}$ is subject to a parent source effect. When other factors are controlled, the mutant areas for all variegating loci in all target organs examined are more extensive when the *Dp* is transmitted through the egg than through the sperm (Spofford, 1959, 1961; Hessler, 1961; Cohen, 1962). Baker (1963) ruled out any mechanism that may depend on prior conditioning of egg cytoplasm by pre-meiotic presence or absence of the rearrangement. He crossed *Dp*/+ ♀ × *W*/*Dp* ♂ and compared *W* and non-*W* sons. Since homozygosity for *Dp* is lethal to males, *W* sons have a maternal, and non-*W* sons a paternal, *Dp*. The latter had 4 to 5 times as much pigment as the former. Lest the difference be attributed to the *W*-bearing and the unmarked third chromosome, the reciprocal cross was also examined, with the same reduction of mutant expression with transmission through sperm.

A parent source effect cannot be clearly found, disentangled from other modifying effects, in Noujdin's (1944) data. Heterozygous daughters of $sc^8/y\ ac$ mothers were as often mosaic when they had inherited their sc^8

chromosome from their father (43·9% from crosses to sc^8/Y) as from their mother (40·0% in crosses to *y ac*/*Y*). However, Prokofyeva-Belgovskaya (1947) found that the distal end of the sc^8 chromosome, comprising 1A–B1 and section 20 in the salivary gland, was "heterochromatized" in 71% of the larval daughters with the paternal rearrangement but in only 20% of the larval daughters with the maternal rearrangement.

Schneider (1962) looked for parental source effects on several rearrangements giving *pe* variegation in *D. virilis*. She found the phenotype most nearly wild-type when the rearrangement was inherited from a homozygous mother. However, the comparisons of heterozygous parents and of homozygous with heterozygous fathers differed from one rearrangement to another, leaving it unclear whether the source of the rearrangement or some other unidentified genetic difference between the stocks crossed was the chief source of the difference. A similar uncertainty exists for a possible parent source effect on the *y*-variegation of $In(1)sc^{S1L}\ sc^{8R} + S$, $sc^{S1}\ sc^8\ w^a\ B$ (*Basc*) reported by Lüning (1954).

It is interesting and I think not irrelevant to note that a parent source effect has been reported for the mouse variegation system cited earlier (Section III.F). Cattanach and Perez (1970) were able to demonstrate for *T*(*1;X*)*ct* that the fraction of pelt in which the c^+ allele, in the inserted duplication, was inactive was higher when the duplication was derived maternally rather than paternally. This was true whether the paternal *Dp*-bearing daughters had had Dp-bearing mothers or not. This finding may be causally related to the visible differences in the degree of condensation of the paternal and maternal sets of chromosomes in colchicine metaphases of the first cleavage of the mouse zygote (Nesbitt and Donahue, 1972). The paternal set was the less condensed, although the quinacrine banding pattern was the same in both sets. In both the mouse and *Drosophila*, sperm maturation includes the replacement of the normal histone components of chromatin by a more arginine-rich sperm histone (Bloch, 1969); there is no similar near-total replacement of the non-DNA components of chromosomes yet identified in oogenesis. This aspect of the different histories of chromosomes in transit through spermatogenesis and oogenesis can be expected to assume importance in a final understanding of parental source effects.

F. Homozygous *vs.* Heterozygous Mothers

In two situations investigated, the variegation was more extreme in offspring of mothers heterozygous rather than homozygous for the variegation-inducing rearrangement. In a number of paired crosses, Noujdin (1944) recorded a higher fraction of mosaicism in offspring of

sc^8/y *ac* mothers than of sc^8/sc^8 mothers, e.g. 6·7% *vs.* 0·6% for sc^8/Y sons, 40·6% *vs.* 13·9% for sc^8/sc^8 daughters, 40·0% *vs.* 4·8% for sc^8/y *ac* daughters. Hessler (1961) found a much higher level of eye pigment in sons of mothers homozygous for $Dp(1;3)N^{264-58}$ than in sons of heterozygotes. Other factors later discovered that might have also differentiated these paired crosses (such as at the *Su*(*var*) locus described in Section IV, G) would have reduced, rather than exaggerated, the magnitude of the difference measured. On the other hand, when I attempted to assess the true magnitude of the effect to be ascribed to maternal homozygosity versus heterozygosity in crosses controlled for the *Su*(*var*) locus (Spofford, 1966), I was unable to demonstrate any difference at all.

G. Other Genetic Modifiers

A number of other, usually less well-defined, differences in the "genetic background" have been contributed by the various stocks whose intercrosses have revealed the types of genetic effect on the variegation phenotype itemized thus far. Allelic differences at loci scattered through euchromatin as well as heterochromatin have been implicated if not thoroughly studied (Schultz, 1950). Some have effects restricted to the variegated expression of particular loci, others to the extent of inactivation for some or many rearrangements. Single variegation-modifying loci have usually been localized only to chromosome, due to the labor of identifying recombinant genotypes that are only revealed in particular variegation assay systems. For example, Hinton (1949) found that the third chromosome from an Oregon-R stock markedly enhanced the eye phenotype associated with *In*(*2LR*)*40d*. The homozygously lethal *E*(*var*)*7* is in 2L (Schultz, cited in Gsell, 1971) although its location is uncertain and in fact it may not even be a single locus. It has a stronger enhancing effect than is associated with most second chromosomes for mottling of *w* and *rst* (Schultz, cited in Lindsley and Grell, 1968), *B* in various B^SY derivatives (Brosseau, 1960) and *y* in the $sc^{8}\cdot Y$ (Gsell, 1971). *E*(*var*)*7* enhanced 12 of the 23 Y-suppressed lethals tested by Lindsley *et al.* (1960). Of these, 11 were also enhanced by $Df(2R)M\text{-}S2^{10}$, which also enhanced 7 of the lethals unaffected by *E*(*var*)*7*.

Certain completely euchromatic rearrangements, *In*(*2LR*)*Cy* and $In(3LR)Ubx^{130}$, are broad-spectrum variegation enhancers (Schultz, 1950, and personal communication; Suzuki, 1965; Spofford, unpublished observations). The particular *C*(*1*)*RM* and *C*(*1*)*RA* chromosomes tested by Suzuki (1965) differed systematically in their effects on the amount of eye pigment developed with $Dp(1;3)N^{264-58}$, *C*(*1*)*RM* associated with the more pigment. It is difficult to assign causes for the effects of these re-

arranged chromosomes; they may have differed in their specific genetic contents as well as in their linear sequences.

One modifying locus that has been given relatively thorough study is *Su(var)*, mapped at 41·4, in 3L (Spofford, 1967). Although it is in the proximal region characterized by low recombination per band, it lies in typical euchromatin to the left of *th*, which has been localized to 72A–B1 (Ward and Alexander, 1957). Most stocks appear to be polymorphic for alleles of *Su(var)*. The studies have been conducted on two extreme alleles, christened *Su(var)* and $Su(var)^+$, which can be maintained in homozygous stocks, although the fertility of *Su(var)/Su(var)* females is low when they are *C(1)RM/Y*.

The spectrum of variegation systems on which the *Su(var)* locus acts, and the roles of the two alleles in these cases, have not been as extensively surveyed as for *E(var)7*. When assayed on the variegated *w*, *rst* or *dm* phenotypes associated with $Dp(1;3)N^{264-58}$ (Cohen, 1962; Spofford, 1967, 1973), or *w* in $In(1)w^{m4}$ or *rst* in $In(1)rst^3$ (Spofford, 1969), the two alleles acted without dominance and with a maternal effect as great as the direct effect on phenotype. *Su(var)* suppressed and "$Su(var)^+$" enhanced. The effects were the most striking with the *w* mottling of $Dp(1;3)N^{264-58}$. A complete range of eye pigment levels could be obtained in *y w/Y*; *+/Dp* males with paternally derived *Dp*, by manipulating the *Su(var)* genotype, from rare traces of pigment in fewer than one in 500 males when both mother and son were $Su(var)^+/Su(var)^+$ to full pigmentation in both eyes of most flies when both mother and son were *Su(var)/Su(var)*.

The temperature sensitivities of $Dp(1;3)N^{264-58}$ stocks homozygous for these two alleles have already been described (Section IV, A.2). It is possible that the *Su(var)* locus is responsible for the elaboration of a diffusible substance. Gearhart and MacIntyre (1971) mentioned that when $Dp(1;3)N^{264-58}$ eye disks were transplanted, the *Su(var)* genotype of the host larva influenced the amount of pigment developed in the disk. Janning (1971) found that the general variegation pattern was intrinsic to the disk in such implants (Section III, F). However, the transplanted disks developed less pigment altogether than appeared in the eyes of normally developing sibs of the donors. Since the host and donor were unrelated, they may have differed in *Su(var)* locus genotype, with consequences mediated by diffusion.

When the two alleles were assayed on *y* variegation in $In(1)y^{3P}$ or *sc* in $In(1)sc^4$, their roles were reversed and the quantitative difference between genotypes lessened (Spofford, unpublished observations). The maternal effect was so slight as to be statistically insignificant. For y^{3P}, *Su(var)* became a recessive enhancer of mutant expression in the post-scutellar bristles. The cores of individual bristles frequently contained intermediate amounts of pigment. They were graded on a scale from 0, for pigmentless,

through 4, for full pigment. The average score for *Su*(*var*)/*Su*(*var*) was 1·4 compared to 2·1 for other genotypes. The average values for backcrosses (1·7) and F_2 (1·9) were close to Mendelian expectations for these segregating sibships. The *Su*(*var*) locus was found to have a non-dominant direct effect on a point mutant at the *sc* locus, sc^2. Each substitution of a $Su(var)^+$ for a *Su*(*var*) gene in the genotype repeatably added an average of 0·5 bristles to the scutellum. The direct effect on the point mutant makes any assessment of the role of *Su*(*var*) in *sc* variegation extremely difficult. Indeed, I have not found it possible to demonstrate any consistent effect of the two alleles at this locus on scutellar bristle number in $In(1)sc^8/Y$ sons of *C*(*1*)*RM*/*Y* mothers. They did have consistent effects on $In(1)sc^4/Y$ sons of *C*(*1*)*RM*/*Y* mothers. Such sons had bare scutellums, but each substitution of a $Su(var)^+$ for a *Su*(*var*) gene in the mother's genotype added an average of 0·06 notopleural bristles to the two sides, and the same substitution in the fly's own genotype added an average of 0·21 notopleural bristles.

Each of the genetic components that has been shown to suppress or enhance variegation in some systems—the Y, the parental source of the rearrangement, $Df(2R)M\text{-}S^{10}$, *E*(*var*)*7*, *Su*(*var*)—either changes the sign of its effect in some others or is ineffective. If any rules govern the type of response a particular variegation system will display to each of these factors, they have yet to be discovered.

A converse question can be asked. Does suppression by extra Y's (especially the part proximal to the KS factors or within the KL factors) and by deleted X's retaining much of their heterochromatin, and enhancement by heterochromatin deficiencies either to the left or right of *bb* in the X, by $Df(2R)M\text{-}S^{10}$, and by haplosomy for the fourth chromosome, guarantee that a particular phenotype is due to a position-effect variegation? The answer most probably is *no*. All these, and a stronger mutant expression at lower temperatures than at higher, have been found for *spa* (Morgan, 1947), a probable point mutant located distally on the fourth chromosome (Hochman, 1971). I have also already mentioned that two mutants that act recessively in females to distort the sex-ratio in their progeny—*abo* and *da*—are less strongly expressed when a Y chromosome is added to the maternal or offspring genotype (Sandler, 1970, 1972). These loci may prove to play a role in the same developmental system that is disarranged in position-effect variegation.

H. Inhibitors of DNA Replication, Transcription, or Translation

Few of the attempts to modify variegation through treatment or feeding of

larvae with substances expected to interfere with mitosis, purine or pyrimidine biosynthesis, faithful DNA replication, mRNA transcription or the translation process have led to interpretable results.

Colchicine suppressed variegation in *T(1;4)*$w^{m258-21}$ females. This may involve simple disturbance of cytokinesis from dissociation of spindle fibres into their component subunits. However, it would be wise to conclude merely that variegation involves at some point a self-assembly process, since colchicine is known to disrupt the self-assembly of other macromolecular systems.

The phenotype of *T(1;4)*$w^{m258-21}$/*w* females was insensitive to a variety of pyrimidine analogues and several purine analogues, but was less mutant with 2,6-diaminopurine or benzimidazole (Schultz, 1956). Azaserine, an inhibitor of purine biosynthesis, and deoxypyridoxidine, among other things an antagonist in serine-biosynthesis, also suppressed variegation. Schultz mentioned that the strongest modifier of *w* variegation was amethopterin, blocking the methylation of deoxyuridine in the synthesis of deoxythymidine (dT). Even wild-type flies showed several anomalies after developing in amethopterin-containing medium. Bristle shape, however, was normal. Hence, the data Schultz presented concerned variegation of the dominant *Sb* allele contained in an insertion of 89B–93D7 into the base of 41A. The percentage of *Sb* bristles decreased nearly linearly with increasing dose of amethopterin. Added dT removed the response to amethopterin while added deoxyadenosine exaggerated it. In interpreting what is possibly a consequence of the inhibition of DNA replication, especially of a region for which thymidine is the limiting factor, it might be well to note that polytenization of the bristle-forming cells occurs early in pupal development and that failure of the *Sb* locus to replicate while adjacent to non-replicating heterochromatin may well be the mechanism of inactivation for this particular phenotype without providing us with much insight into the general process of variegation.

A remarkable insensitivity has been displayed to inhibitors of transcription and translation. Baker (1967) administered actinomycin-D, puromycin, cycloheximide and 5-methyl tryptophan separately in the food to first instar *Dp(1;3)*N^{264-58} larvae without effect on the eye pigment level in the resultant adults, except for a slight increase in drosopterin with the last agent, perhaps through a direct effect on the pteridine pathways. For some of these inhibitors, the insensitivity may result from impermeability of the eye disk to them. Kuroda (1970) noted that explanted eye disks differentiate ommatidia when provided with appropriate hormones *in vitro*, whether or not bromodeoxyuridine, actinomycin-D or puromycin are present. Either the appropriate macromolecules had already been synthesized by late third instar, or the inhibitors had not reached their target sites in the cells.

However, in the case of actinomycin-D, larval growth was arrested at the end of the first instar and the number of cells in the eye-antennal disk remained static through a time interval during which ,with progressively later induction of somatic crossing over, the size of twin spots decreased and their number increased at the same rate as in the untreated controls (Perez-Davila and Baker, 1967). Measurement of the DNA content of the eye disks after growth had been arrested for a day indicated the possibility that some DNA replication had continued (Baker, 1968). In that event, when growth resumed following removal of inhibitor, the recombined strands could sort out as cytokinesis caught up with the earlier DNA replication. What was demonstrated concerning the process of variegation was that the stable inheritance of the tendency to later activity or inactivity of the *w* locus that had been determined earlier (Section II, F) did not require mRNA synthesis during early second instar, or if it did, transcription of the relevant loci was less sensitive to inhibition than is the case for most loci.

V. Heterochromatin and Chromosome Organization

The subject of heterochromatin—its definition, its chemical peculiarities, its distribution along the chromosome axis in the different cell lineages through the development of the fly, its transcriptional inactivity, its replication time during the cycle of the dividing cell—is more fully treated elsewhere in this volume. I shall focus only on those aspects that seem particularly relevant to an understanding of the mechanism of variegation.

A. The Time of "Heterochromatization"

It is well to recall that a segment of a chromosome may be heterochromatic in one kind of cell, but not in another. In fact, during cleavage, no part of the chromosome complement is heterochromatic in the sense of remaining compactly coiled during interphase. Not even chromomeres can be distinguished. The absence of chromocenters in cleavage nuclei was first noted by Huettner (1933). Heteropycnotic regions begin to appear during the prophases of the second hour after egglaying. Typical chromocenters and nucleoli are formed during the first prolonged interphase while the long postponed cytokinesis at last forms the blastoderm (Rabinowitz, 1949; Mahowald, 1968). At this time, not only do larger extents of the chromosome fiber condense into a tangled and apparently disorganized mass in the heterochromatic regions, but short extents, of moderately varying length at relatively uniform intervals from each other condense into what may be chromomeres (Ashton and Schultz, 1971 and personal communica-

tion). Subsequent mitoses have both a longer interphase and a longer prophase than the syncytial cleavage mitoses.

When a 160-minute lateral blastoderm nucleus, with well-developed nucleolus and heteropycnotic clumps, is injected into an unfertilized egg, the first 10 minutes are occupied with enlargement and rounding out of the injected nucleus, loss of heteropycnosis, and recovery of the homogeneously diffuse appearance typical of an interphase cleavage nucleus. In 5 more minutes the nucleolus disappears and, 20 minutes after injection, the nucleus divides (Illmensee, 1972).

Thus, whether a given chromosome region is to be heterochromatic or euchromatic at a particular time in a particualr cell must be determined by factors some of which are extrinsic, some intrinsic to the coded base sequence in the DNA of the chromosome itself. In dividing cells, the condition of heterochromaticity is not irreversible for a given chromosome region although it appears to be inherited mitotically with considerable faithfulness. Whether precisely the same segments of chromosome are included in heterochromatin in most tissues is not known.

In the neuroblast and germ-line cells of most *Drosophila* species, heterochromatin is localized at the centromeric end of the chromosome arms, as it is in many eukaryotes. Although rearrangements can and do move large blocks of heterochromatin distally between stretches of euchromatin, this condition does not seem to be evolutionarily stable. The heterochromatizable portion is larger for the sex chromosomes than for the autosomes, with the exception of the "dot" chromosomes in some species. The Y chromosome is completely heterochromatic, except for the nucleolus organizer and, occasionally, the regions seen as "constrictions", in most cells. Yet it becomes diffuse and is transcribed during prophase in the primary spermatocyte and is probably diffuse in the nurse cells of the ovarian follicle of XXY females (Schultz, 1956). About one-third of the X chromosome is heterochromatic in most cells, but the whole chromosome condenses earlier than the others while the Y is active during spermatogenesis. Lifschytz and Lindsley (1972) propose that the condensation is initiated in a heterochromatic region between *su(f)* and the nucleolus organizer, for which many deficiencies and translocations to autosomal euchromatin are male sterile.

The above information has pertinence as to whether we should expect a given rearrangement, involving a heterochromatic breakpoint, to induce variegation of a given locus. If the locus were to act, once and for all, during the cleavage stages, it should be incapable of position-effect variegation. If it acts in a cell-lineage at a time at which the "heterochromatic" region to which it is coupled is also active, there should be no variegation for that locus in that cell lineage regardless of its variegation in

other tissues. The earliest time at which a locus could be heterochromatically inactivated, and thus subject to variegation, is during blastoderm formation.

B. The Chemistry of "Heterochromatin"

1. Peculiarities of DNA

Inhomogeneities of two sorts have been detected among relatively short fragments of physically disrupted DNA from chromosomes. One sort of inhomogeneity concerns the base sequence, regardless of its average content of the four bases. Some segments include the same relatively lengthy base sequences that are found on segments from other parts of the chromosome set—these are the "repeated sequences" of "repetitive DNA". Other segments contain only unique base sequences.

The other sort of inhomogeneity concerns the average base composition of the segment. When an appreciable fraction of all the segments share a common base composition, sufficiently divergent from the average for the whole genome, a "satellite peak" forms during CsCl density gradient centrifugation. The fraction of guanine-cytosine (GC) base pairs in the DNA is linearly related to the density of the set of segments forming the satellite. It is also linearly related to the melting temperature of the native fragments or of the corresponding sections in unfragmented chromosomal DNA. Although satellite DNA would be expected to include more than its share of repeated sequence DNA, it may be anticipated to include some unique sequence DNA as well. Some repeated sequence DNA should also be expected to have a "typical" base composition and be included in the "main band" upon centrifugation. These expectations have been confirmed for the DNA of a number of higher vertebrate species, both before and after separation of densely condensed from diffuse chromatin (Comings and Mattoccia, 1972).

In *D. melanogaster* the overall base composition of the two highly heterochromatic sex chromosomes in adult cells is within narrow limits the same as for the autosomes (close to 60% AT) since no systematic differences were found in comparisons of whole DNA from XO, XY and XYY with XX and XXY (Perreault *et al.*, 1968). Nevertheless, when smaller segments of DNA are examined, some differences appear.

One satellite DNA, virtually pure double-stranded poly-deoxyadenylate-deoxythymidylate (dAT), has been found in *D. melanogaster* and *D. virilis*, both as a small plateau in the melting curve of nuclear DNA from embryos, and in CsCl gradients (Blumenfeld and Forrest, 1971). It constituted 3·8% of the DNA of embryos from the Oregon-R stock, males and

females pooled. The amount varied approximately linearly with the amount of Y chromosomal material in the pooled genotypes of the two sexes in stocks differing in the number of extra arms of the Y carried in one or both sexes. From the increment in % dAT per whole additional Y, it was possible to infer that roughly half of the dAT in a normal chromosome set is on the Y, distributed on both arms, the remaining half somehow apportioned to the X and autosomes. The proportion of dAT in pupae and adults is half its value in embryos (Blumenfeld and Forrest, 1972), indicating underreplication. Ellison and Barr (1971a, b, 1972) have strong presumptive evidence that regions of nearly pure dAT show an extraordinarily brilliant fluorescence after quinacrine staining. The metaphase chromosome pattern of intense fluorescent spots differs between species. In *D. melanogaster*, the entire Y, the basal third only of the X heterochromatin, most of the fourth chromosome, and a very small part of the metacentric autosomal heterochromatin, not immediately next the centromere, fluoresced intensely. Most was not replicated during polytenization in the salivary gland. In salivary gland chromosomes, bright flecks could be seen in the chromocenter, and bright bands in 81F, at the base of 101, and in 102D1–2. Polymorphism exists for brightness at 83DE, a "weak spot". Ectopic pairing was common between 81F and the slightly more DNA-rich 83DE region when the latter was bright but very rare when the latter, less DNA-rich, was not bright (Ellison and Barr, 1972). The polymorphism may be for the dAT material itself in 83DE but is more likely for the capacity of some dAT material at that site to replicate as the chromosome polytenizes. The fact that 83DE is a constriction easily broken as the chromosome is stretched suggests the normal presence there of non-replicated chromatin. The dosage of some component in this region is extraordinarily important. This region was not only one of the few haplo-lethal regions discovered by Lindsley *et al.* (1972) in their survey of *D. melanogaster* autosomes, but it was the only triplo-lethal region.

Another low-density satellite with about 70% AT constitutes nearly 8% of the DNA of larval brain and imaginal disk tissue in both *D. melanogaster* and *D. virilis* but is virtually absent in salivary gland chromosomes (Gall *et al.*, 1971). This satellite is not homogeneous in base sequence in *D. melanogaster* since when it was dissociated into single strands, at least two sets of complementary light and heavy strands could be separated by their densities. However, radioactive RNA transcribed *in vitro* from this satellite annealed only to the chromocenter of salivary gland chromosomes (Rae, 1970). There was a similar localization of the repeated sequences of a slightly heavier, but still AT-rich, satellite of *D. virilis* (Gall *et al.*, 1971). The amount bound in the chromocenter of the polytene nuclei was the same as for a diploid nucleus under conditions in which only repeated

sequences would anneal. Hennig (1972a) found both a high-GC satellite and a roughly 70% AT satellite in *D. hydei*, whose amount depended more on the number of Y chromosomes than of XR's, although XR has as much DNA as the Y and together they account for half of the heterochromatin DNA in this species. However, only a fraction of these satellites could have been composed of repeated sequences. The total proportion of DNA in repeated sequences, regardless of base composition, correlated well with the number of XR's but not at all with the number of Y's. As much as two-thirds of XR, but no more than a quarter of the autosomal heterochromatin and very little of the Y chromosome could be repetitious DNA. This, although the Y contains regions both unusually high and unusually low in AT base pairs.

Repeated sequences of main-band average base composition have been localized in both *D. melanogaster* (Rae, 1970) and *D. virilis* (Gall *et al.*, 1971) salivary gland chromosomes. In *D. melanogaster*, they are most heavily concentrated in the quasi-heterochromatin of the arm bases but are scattered throughout the euchromatic arms with occasional local concentrations, as near the tip of 2L. One moderately repeated sequence anneals to at least fifteen bands in four different chromosome arms and the quasi-heterochromatin (Wensink *et al.*, 1974). In *D. virilis* they are not especially concentrated toward the chromocenter but are more heavily distributed along the X chromosome than the autosomes.

Thus "heterochromatin" cannot be characterized by a particular base composition or as the sole repository for repeated sequences. Heterochromatic regions differ among each other more sharply than do euchromatic regions of similar length in base composition and are more often AT-rich, including regions that are virtually pure poly-dAT. Repeated sequence DNA of extreme base composition is therefore more likely to be confined to heterochromatin. However, repeated sequences of average base composition are found in both heterochromatin and euchromatin, and a substantial part of heterochromatin consists of unique sequence DNA.

There is good reason to believe that the highly repeated sequences alternate with unique sequence DNA in heterochromatin. When chromatin DNA from late embryos was sheared to successively smaller size, segments melted, and reannealed so briefly that only very highly repeated sequences would reanneal, an interesting segment size dependence was revealed (Kram *et al.*, 1972). Reannealed DNA, separated from single strand DNA on hydroxyapatite, had two peak densities—one slightly denser than whole-chromatin native DNA, whose amount fell as the segments became shorter, and the AT-rich satellite, whose amount rose until it was the only very rapidly reannealing material when segments were only 500 bases long. From the relationship between the ratio of the two peaks and segment

length, Kram *et al.* argued persuasively that unique sequence segments of 3000 to 6000 base pairs separate 2500 to 10,000 nucleotide-long regions of highly repeated sequences so that the larger segments recovered usually contained both reannealed and single stranded parts.

2. *Histones*

Oliver and Chalkley (1972) have resolved 10 histone fractions in adult *D. melanogaster* chromatin. No qualitative differences in the histones have been found between non-polytenizable heterochromatin and the remainder of the chromosome complement of *D. melanogaster*—the positions of the bands in acrylamide gels after electrophoresis of histones from salivary glands and from imaginal disks were the same (Cohen and Gotchel, 1971). There may yet prove to be differences in total amount or relative proportions of the individual histone fractions between heteropycnotic and diffuse chromatin. Berlowitz (1965) found 2·6 times as much histone per DNA in the heterochromatized paternal set as in the euchromatic maternal set of chromosomes in the mealy bug. Polylysine and lysine-rich (F_1) histone have a particularly high affinity for AT-rich DNA (Georgiev, 1969). There may also prove to be differences in the extent of acetylation, phosphorylation, and S–S crosslinking of various histones in the two kinds of chromatin, acetylation, phosphorylation, and reduction of S–S bonds having been related in other organisms to preparation for transcription or replication (reviewed in Wilhelm *et al.*, 1971)

3. *Non-Histone Chromatin Proteins* ("*NHP*")

These usually constitute a somewhat smaller portion of the proteins in chromatin than do the histones. They are a very diverse assemblage of proteins, sharing the property of binding closely to DNA. Since they contain more acid than basic residues, they have greater selectivity for the base sequences with which they will bind than do the histones, which are presumed to form salt linkages with the phosphates in the DNA backbone. The histones can be viewed as a set of general-purpose "clamps" for retaining a particular tertiary configuration of the DNA double helix. The NHP's by their binding determine the specific configuration. Both NHP's and histones form a sort of self-assembly system when combined with DNA. This was elegantly demonstrated in work in other organisms reviewed by Paul (1970). The tissue-specificity of RNA transcription and also a normal chromatin appearance in electron micrographs was restored to DNA to which the NHP and histone, previously removed, were added back, in that order, *in vitro*.

It is presumably the differential distribution of certain NHP's that gives rise to the characteristic dark (non-fluorescent) bands in the quinacrine-stained mammalian chromosome. This banding pattern is constant throughout development in a diversity of tissues (Nesbitt and Donahue, 1972). Pronase removes these bands, leaving the entire chromosome stained uniformly though not brilliantly fluorescent (Comings, 1971).

The specificity and large numbers of NHP's make them excellent candidates to be the chromosomal constituents that differentiate condensed heterochromatin from diffuse euchromatin. Because they form self-assembly systems with DNA, one might expect their association with DNA to be sensitive to agents known to disrupt other self-assembly systems. If some heterochromatin-specific NHP's differed in sensitivity from NHP's in general, heterochromatic condensation might be inhibited. Two instances of influence of self-assembly system inhibitors on heterochromatin have been reported. Low temperature inhibited heterochromatin condensation in *Vicia faba* (Caspersson *et al.*, 1968). Colcemid applied in G2 inhibited the condensation of the centromeric heterochromatin in Indian muntjac fibroblasts (Comings, 1971). The usually heterochromatic region, identified by lack of separation of the chromatids, was longer and thinner than normal, while the euchromatin, with separated chromatids, became progressively shorter and more compact.

To some extent, there may be common factors in the mechanism of any form of chromosomal condensation. NHP's are implicated in the condensation of the chromosome fiber into chromomeres and their unravelling during activity. The NHP:histone ratio rises before transcription levels are heightened, such as prior to puffing in an ecdysone-treated salivary gland chromosome band (cited in Spelsberg *et al.*, 1972). The S–S:SH ratio rises in NHP's as well as in the F_3 histone fraction as the chromosomes condense during prophase. And in the heteropycnotic regions of the interphase nucleus there is less than a third as much phosphorylated NHP per unit DNA as in the diffuse regions, with a comparably low rate of phosphate turnover. NHP synthesis continues throughout the cell cycle and not just during S, as is the case for histones.

In short, while little is known about the NHP's, and no single NHP has been isolated and characterized, one can hope that their study will elucidate many aspects of organized chromosome behavior and in particular, heterochromatization and position-effect variegation.

C. Timing of Heterochromatin Replication

During the cleavage mitoses, DNA synthesis proceeds throughout the

brief interphase, at a pace that can be accomplished only if the individual replicons are much smaller and correspondingly more numerous than later in development (Rudkin, 1972 and Blumenthal *et al.*, 1974). With the extended interphase accompanying blastoderm formation, not only do heteropycnotic regions appear in the chromosomes, but the S-phase is greatly extended, replication of the heteropycnotic regions being deferred till near its end. Breviry of the cleavage synthesis phase and its extension with late replication of heterochromatic regions is common to many animal zygotes (Brown and Dawid, 1969), exceptions to the rule that centromeric heterochromatin is late-labeling being uncommon (Comings, 1972). Whole chromosomes that become facultatively heterochromatic—the second X in mammals of the paternal set in mealy bugs—become late-replicating at the same time (Brown, 1969). Late replication has thus come to be considered a hallmark of heterochromatin.

The replication pattern has been studied in larval neuroblast cells in the large-chromosome species *Samoaia leonensis*, a close relative of the genus *Drosophila*. Following the termination of all tritiated deoxythymidine (^{3}HdT) incorporation in euchromatin, labeling continued in the regions intensely fluorescing with quinacrine stain: the dot chromosome, the autosomal arm bases, the short heterochromatic base of XL, and the whole of XR (long as in *D. hydei*) and the Y (Ellison and Barr, 1972). The dot chromosome heterochromatin completed replication first. The long autosomes and XL finished next, XR and the Y last. It may be noted here that while *S. leonensis* heterochromatin is rich in poly-dAT, it incorporated virtually no tritiated uridine (^{3}HU) in Malpighian tubes (Kessler *et al.*, 1973), confirming the transcriptional inactivity of at least one component of heterochromatin DNA in one drosophilid species.

Even in neuroblasts in *D. hydei*, Berendes and Keyl (1967) showed that the chromocentral regions were not merely replicated later, but were underreplicated.

With the greater detail accessible to study in the salivary gland chromosomes, some individual bands have been found to require nearly as long to replicate as the quasi-heterochromatic arm bases. These all contain one or more large bands, such as are in 58A, 59D, 11A, 12DE, 3C, or 1A in *D. melanogaster* (Plaut, 1969; Rudkin, 1972) or 16D in *D. hydei* (Berendes, 1966). Some of these long-, and thus late-, replicating bands are in constricted regions where the chromosome breaks easily upon squashing, as in 16D of the *D. hydei* X, just proximal to the white-Notch region. Rudkin has concluded that these long-replicating bands contain unusually long replicons. A long replication period should not by itself be considered sufficient evidence for the quasi-heterochromatic nature of these bands, but should be duly considered in assessing the possible linear heterogeneity

of chromatin type within the large regions grossly classed as euchromatin (see Section V, D).

Rearrangements involving breakpoints in late-replicating regions often do not result in detectable alteration of the replication-pattern of the newly adjacent regions in the salivary gland chromosomes. That is, when the euchromatic region is visible, it incorporates ^{3}HdT at the same time as the homologous region in its normal position, shown first for *T(1;3)ras*^{V} (Barr *et al.*, 1968). Since the region can be scored as replicating or not only when it is visible, i.e. not "heterochromatized", this finding is not as decisive as it might otherwise seem.

The euchromatic tips of longer free duplications that are more easily scored—*Dp(1;f)*'s that are paired with the basal end of the normal X—have been shown to replicate asynchronously with the homologous part of the normal X. The replication times of 1AB in the normal X and in *Dp(1;f)AM* are negatively correlated (Bender *et al.*, 1971). Replication in *Dp(1;f)R* is later than in the normal tip (Ananiev and Gvozdev, 1974) in some cells of the salivary gland, under conditions that enhance variegation, leading to underreplication near the breakpoints, and undertranscription through most of sections 1B–2F.

Normally late-replicating regions are also affected in some but not all rearrangements. Roberts (1972) reports that 101D3-4 replicates in synchrony with the chromocenter even after translocation distal to 100B. Barigozzi *et al.* (1969) found that in embryo tissue culture both parts of the Y chromosome retained its normal lateness in *T(Y;2)B/b*, a reciprocal translocation to 2R identified in salivary gland chromosomes as proximal to banded region 41. A distal tip of the Y capping 88C in *T(Y;3)P80* replicated extraordinarily late, while in the reciprocal Y^P 3^D chromosome, the remainder of the Y replicated rather earlier than usual. In another rearrangement, one Y fragment was a ring that retained the normal replication time. The remainder of the Y was inserted into X heterochromatin; it replicated as early as euchromatin.

D. Intercalary Heterochromatin . . . ?

Whether the chromosome fiber carries segments of DNA that become heterochromatic in some cell lineages sandwiched—intercalated—in the midst of fiber whose only condensation is into chromomeres has been a long-debated issue, chronicled by Hannah (1951) and Lefevre and Green (1972). Many criteria have been proposed (Prokofyeva-Belgovskaya, 1941) to locate the positions of intercalary heterochromatin on the salivary gland chromosome map. Some of these criteria, such as existence of cytological repeats and "high breakability", are probably improperly

applied. Regions identified as heterochromatic because X-rays induce in them a high number of breaks per band (Kaufmann, 1946) are usually regions with dense bands. The breakage frequency correlates remarkably well with the amount of DNA in the region (Lefevre, 1969). Yet some of the dense-banded regions highly breakable by X-rays, e.g. 3C, 11A, 12DE and 19E, have deep constrictions such as would result from a lesser or non-replication of an interval in the chromonemal continuum adjacent to these intervals of prolonged replication and great compaction into dense bands.

Ectopic pairing has been used as a clue to the heterochromaticity of regions, whether centromeric or intercalary. The ectopic pairing between the quinacrine-bright spots in 83DE and 81F must signify strong similarity and probably homology of DNA sequence, since the brightness is associated with the presence of poly dAT (Barr and Ellison, 1972). Kaufmann and Iddles (1965) have published a detailed map recording all the ectopic associations of bands in 400 cells. Dense bands were responsible for most of the ectopic pairings. For example, region 3C1–3 was recorded as pairing at least once each with 3C5–7, 4D1–2, 10B1–2, 11A, 56AB, 96A and 98C. Pairing with the zeste band 3A3 was not noted, nor was the 81F–83DE association. However, included among the regions most often paired ectopically are the "discontinuities" or offset constrictions in 11A, 12DE, 19E, 33A, 35E, 36DE, 42B, 64C, 70BC, 75C and 89E.

Another criterion for intercalary heterochromatin could be its induction of position-effect variegation in a locus whose expression can be unambiguously mosaic, such as white. By this criterion, the 5S RNA locus in 56EF might be in or near intercalary heterochromatin. One of the few puzzling instances of white variegation is provided by $T(1;2)w^{13G2}$, placing the *w* locus in 56F.

The value of these various clues could be checked against the properties of sections of centromeric heterochromatin that have been known to be inserted into euchromatin through rearrangements such as $In(1)sc^{8}$, or the partial reversions of $T(1;4)w^{m11}$, or $In(1)lz^{S}$. They retain their refractoriness to crossing over (Baker, 1958) and often evoke or continue to evoke variegation (Panshin, 1938; Hannah-Alava, 1971; Baker, 1971; Breugel, 1970). They tend to pair ectopically with the chromocenter (Prokofyeva-Belgovskaya, 1947; Hannah-Alava, 1971; Breugel, 1972). In salivary gland chromosomes the inserted heterochromatin is itself not visible. Sometimes its position is occupied by a normal-appearing interband, sometimes by a constriction or an abrupt change in chromosome diameter. When the distal heterochromatin includes the nucleolus organizer, this is sometimes said to be represented as a puff rather than a fully devleoped nucleolus (Hannah-Alava, 1971; Breugel, 1972). The banding in adjacent euchromatin becomes

variable in appearance, interbands occasionally missing, with perhaps even some underreplication of euchromatic DNA. $In(1)sc^{8}$, in which most of the centromeric heterochromatin of the X, including the nucleolus organizer, is brought next to 1B2, is characterized by extreme variability in the distinctness of banding not only of 1AB but also of section 20, where Prokofyeva-Belgovskaya (1947) could never distinguish more than subsections ABC. In the shorter inversion $In(1)w^{m4}$ not including the nucleolus organizer, the whole of 20A–F can be seen inverted (Prokofyeva-Belgovskaya, 1947; Schalet and Lefevre, 1973). An insertion of polytenizable but variably banded 41B–F between 3C5 and 4C4 on the X led to more frequent clear banding of part of the inserted material ($T(1;2)w^{m258-36}$, Sutton, 1940), as well as variegation of *w* and *rst* distal to the insertion.

It must be mentioned here also that suppression of gene activity has several times been attributed to adjacent insertions of material behaving like variably polytenizable heterochromatin of uncertain origin. Both bw^{D} (Hinton and Goodsmith, 1950; Slatis, 1955b) and ey^{D} (Hochman, personal communication) have been so explained. Slatis found the element in question to be invisible in some cells but to vary in distinctness in others. At most he could distinguish three thick and one thin band, suggesting that the source of the insertion included the occasionally banded region bordering the salivary gland chromosome euchromatin. Thirty per cent of the cells showed a distortion or break between this element and 59E1–2 as if the insertion was considerably underreplicated.

As must be apparent in the foregoing presentation, I consider evidence of non-replication the best guide to the existence of heterochromatization of a section of the chromosome fiber in the midst of euchromatin in the salivary glands. Underreplication or erratic band formation, comparable to that normally seen at the bases of chromosome arms, can perhaps indicate proximity to a truly heterochromatic, hence non-polytenized, section. The other criteria, although applicable to material adjacent to heterochromatin, do not differentiate the smaller chromosome fiber knot that is called a large chromomere from its very much more massive entanglement in the heterochromatin of a diploid nucleus, or in the virtually unreplicated material in the chromocenter of a polytene cell. By the more stringent criteria, there remain several regions in the normal chromosome that may contain intercalary heterochromatin. I am not aware that any of these, except possibly 56EF, have been implicated as variegation inducers.

VI. Summary and Conclusions

Heterochromatin is chromosomal material that is condensed throughout most of the cell cycle and that is transcriptionally inactive. Certain

chromosome regions are heterochromatic in nearly all cells throughout most of their development. There is no present reason to suppose that the common behavior of these regions reflects an identity in their molecular composition—indirect evidence indicates at least a certain degree of linear differentiation within heterochromatin.

From the spread of heterochromatic properties to normally euchromatic chromosome regions in euchromatin-heterochromatin rearrangements, it must be concluded that the normal boundary zones between euchromatin and heterochromatin have special properties in limiting the extent of the chromonemal thread that is condensed and transcriptionally inactive. Some of the genes in these boundary zones must be presumed to require these special circumstances for function, since they become inactive when moved into more distal euchromatin, and sometimes also when moved to certain different associations with centromeric heterochromatin. From a detailed study of the relation between the various chromosome components and factors that influence the extent and frequency of inactivation of euchromatic regions juxtaposed to particular sections of heterochromatin, it may be hoped that a molecular explanation of the normal heterochromatization as well as the variegation process will emerge.

Acknowledgement

I am indebted to Drs. Aloha Hannah-Alava, George Lefevre, Jr., and Bernard Strauss for their critical reading of this manuscript. They are in large part responsible for whatever clarity it has.

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Note added in proof, see page 1429.

Notes added in proof

Chapter 24 Position-effect Variegation in *Drosophila*

Janice B. Spofford

(1) Since my chapter was written the important work of Peacock *et al.* (1974) has been published, describing the isolation and characterization of five DNA satellites accounting for much of the non-polytenized heterochromatin DNA. Most of these satellites form extremely long tracts. Four are also located in 21CD of 2L. Sederoff *et al.* (1975) find tracts containing roughly 750 repeats of a five base-pair sequence in which one strand contains only pyrimidines and its partner only purines. These tracts are scattered in the heterochromatin of the Y and second chromosomes.

(2) Hoechst 33258 stain produces a still more brilliant and differentiated fluorescence pattern in prometaphase chromosomes (Holmquist, 1975). The heterochromatic bases of all four long autosome arms are clearly distinguished. At least eleven regions can be discerned in the Y chromosome. The intensity of Y chromosome fluorescence permits its identification in polytene nuclei, usually adjacent to the base of 2R.

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