CHROMOSOME CYCLES

In typical asynaptic cells, every mitotic division is preceded by complete uncoiling of the nuclear DNA for equal segregation to the two daughter cells. During a normal mitotic cycle, complete chromosome replication is sequentially followed by chromosome condensation, kinetochore separation, nuclear division and finally, the cytokinesis. In contrast, completion of a cell cycle after partial or complete chromosome replication but prior to the subsequent events leads to increased normal DNA content and specific patterns of genome organisation depending upon the point at which the cycle is "short circuited". Thus variations like polyteny, polyplody and cytokinesis stage result from arrestment of the basic mitotic cycle at various points.

In different cell types of Drosophila, polycyst and polypolyne type chromosome cycles are seen in which the celluar events prior to kinetochore separation. To avoid ambiguity, it may be noted that these two terms are being used here to denote specific types of mitotic organisation as defined by Wiegler et al. (1). Thus polyteny implies a specific nuclear differentiation in certain cell types of some insects, prototypes and amphioptera represented by the presence of banded polyteny chromosomes. The term polyplody is used to designate either the condition of multistranded (polysome) metaphase chromosomes or the condition of endoreplication cycles giving rise to interphase nuclei with increased DNA content but with no discrete banded chromosomes of chromosome regions.

POLYPOLYNE REPLICATION CYCLES IN DROSOPHILA

Polypolyne chromosomes are found in a variety of larval and adult cell types of Drosophila. The best well known are those from the larval salivary glands. The replicative organisation of polypolyne chromosomes has almost exclusively been studied in larval salivary glands, the basic features of which are summarised below (also see ref. 2). In polypolyne nuclei the basic chromosome fibril replicates repetitively and the daughter strands remain Relatively extended but their precise lateral accretion results in the well known banding pattern of these chromosomes. It is now well established that the entire length of the basic chromosome fibril of Drosophila chromosomes does not replicate equally during polypolyne replication cycles; at least three, regularly spaced, different levels of DNA replication are known (3,4). The role of the pericentromeric heterochromatin which is enclosed in highly repetitive and/or satellite sequences as the a-heterochromatin in the chromosome region of polypolyne nuclei (5-9). The chromosomal clusters, located within the a hetero-
chromosomes regions of mitotic chromosomes, show another level of nu-
comal replication since sequences polytene sut few cycles than the bulk of metaphase, which provides the 3rd level [10,11]. The replication cycles of the phasemate oocytes, labeled withi
the body of the midzone of polytene nuclei, may have been proposed to be unequal [12] alone the relative DNA content of consecutive band and interband regions have been found to be differ so that certain bands may have replicated more often than others. Our recent studies using B4B fibre autoradiographic sequence have pro-
vided some evidence on this aspect [13, and other unpublished data]. In agreement with the results of another recent B4B fibre autoradographic study by Schambon [16], we find at least two ma-
jor categories of replication organization in polytene nuclei: one group being characterized by a large replication zone (about 30%) and a faster rate (3µm/min) of fork migration while the other group has shorter (about 10%) regions and a slower rate (1µm/min) of fork migration. We have also obtained evidence which suggests that the first group of replicons is active in the early part of a poly-
tene period and the second group (nuclei) during the later part than heterochromatic centromeres and certain band regions [15] are replicating. It appears to us that this dual replication organization is related to the unusual polytization of different band interband regions as proposed by Lee [14]. This aspect will be discussed in detail elsewhere.
The most significant point that emerges from the above features of polynucleo replication cycles is that different dna sequences, even those located on the same chromosome and thereby expected to be part of the same long DNA molecule [15] participate unequally in polytene replication cycles. Obviously, the peculiarities of these sequences in the two different regions result in different photocopying patterns, which is an interesting aspect. It is interesting that in these nonreplicating interphase nuclei also different dna sequences replicate unequally. The first evi-
dence of this phenomenon was observed in hetero- and euchromatic regions in larval brain cells using the cytophotometric analysis of Herbert and Kops [15]. These data showed that many of the larval brain cells in XoB have more DNA than expected in a main cell population but not in the larval brain cells in constant in hetero- and euchromatinic regions of a nucleus which was found to be the case, the heterochromatic chromosomes usually showing lower
creased. This indicates that in larval brain cells, the hetero-
and euchromatin are not necessarily correlated. It may be
that the euchromatin is actually the more active region, and
the heterochromatin is the more inactive region. However, this
may be due to the fact that the euchromatin is more likely to be
active in the larval brain, while the heterochromatin is more likely
to be inactive. Therefore, the presence of euchromatin in larval
brain cells may not necessarily predict the presence of
heterochromatin in the same cell. In general, the presence of
heterochromatin may indicate that the cell is less-active, while
the presence of euchromatin may indicate that the cell is more-active.

In conclusion, the presence of euchromatin in larval brain cells
is a strong indicator of cell activity. The absence of euchromatin
may indicate that the cell is less-active or inactive. However, it is
important to note that this is not always the case, and other factors
may also contribute to the activity of the cell. Therefore, further
research is needed to better understand the role of euchromatin in
larval brain cells.
Earlier cytophotometric studies (2) suggested an increase in the NAD content in metaphase cells to the final larval brain over those in the 1st instar larval brain and this was implied to indicate increase in the lateral striatum with increasing larval age (21). These results, however, have not been confirmed in a later study (24). We have been studying the 3rd instar larva of *metaphase* development in brain cells of *Drosophila* larvae for the past few years (25,26) by cyto-morphology and *Trypsin*-staining and *Trypsin*-autofluorescence techniques. Our results have confirmed the interpretation that while the undifferentiated early-senior cells of *Drosophila* bear undifferentiated, in late larval brain cells, the *titin* and *titin*-like structures. This aspect has been discussed in detail elsewhere (27,28).

On the basis of above, we believe that in brain tissue of *Drosophila* larvae, repeated autophagic cycles may be periodically mono- or super-aggregated by chromosomal condensation and active division cycle so that polysemous interface membrane give rise to 'polysemous' metaphase with polysemous chromosomes. In this context it may be said if the autophagy cycles which decide or watch over to a mitotic division are only those in which the hetero- and autoreactive regions both have replaced immediately prior to mitosis or other cells may also come to autolysis. I will present here some results which suggest that the undifferentiated interface membrane in which the hetero- and autoreactive regions have replicated in independent cycles, and possibly uniquely, also enter autolysis. These studies have been done with brain ganglia from late 3rd instar larvae of *Drosophila*. The *posito* larval autophagy (28,29) in this studies includes 2 periods of larval chromatin (neurogenesis to pro-metaneurite I, metaneurite IIa and pro-metaneurite Ia) and a pair of small late-like chromatin; the Telecerebroencephalon to mate to a sub-senior-linked sub-metaneurite IIIa, IIb, and the autoreactive chromatin simultaneously (25,26). All chromatin, whatever the tiny, terrapituitary granule heterochromatin blocks (see Fig. 3). The second larval brain ganglia were cultured in vitro (30) for labeling with "T-thymidine. In one set, rapidly mitotic ganglia were pulse labeled with "T-thymidine (24h/4h/sorption, 1:6 C02/4h, 30% for 30 min), immediately fixed in fix (1:1:1 sox:ethanol:acid) and air-dry prepared (25) for autoradiography. The second set, slowly mitotic ganglia were cultured in the presence of "T-thymidine (30h/4h) and air-dry prepared (24h/4h/4h) for 30h of cult. In the 3rd instar larval ganglia were cultured in the presence of "T-thymidine (30h/4h) for a period of 30h of which the first 15h were without and the last 15h were with colchicine (0.02%). Since the replication cycles of different cells in brain ganglia of normally growing 3rd instar larvae are highly asynchronous in a single series of experiments, an attempt was made to synchronize their replication cycles by feeding late 3rd instar larvae on 5-Fluorodeoxyuridine (5FU), supplemented salt (CoA) and Fe(II). It has been shown that after 45h Fe(II)-feeding, the replication cycles in nearly all cells in brain ganglia are blocked at the beginning of the S-period (25,26). For the present study, in the initial experiments the larvae were fed for 45h; results suggested that the
Fig. 1. Metaphase chromosomes from late 3rd instar larval brain ganglia of *Drosophila*. Note the dark stained heterochromatin regions of all the larger chromosomes. x 1000.

Fig. 2a-g. Patterns of $^{3}H$-thymidine labelling of larval brain interphase nuclei: a) un.injected ganglia palae labelled; b) Th in vivo PHH-synchronized ganglia palae labelled; c) PHH-synchronized ganglia chronically labelled for 24 h; d) Unlabelled; e) chromosomem labelled; f) neuronactin labelled; g) both labelled. x 1000.

Fig. 3. A cluster of metaphase in brain ganglia exposed to thiobenzamide in vitro for 24 h. x 1000.
Fig. 5. Metaphase in waromotilized brain peeples labelled chromically with Br-Thymidine for 68 (a-e) or 24h (f-g). 
- A of procentahse end a ch centphase with only the hentro-
chromatin regions labelled. 
- The same plate after removal of silver grains. 
- A ch centphase with heavy labelling of heterochromatin of both chromosomes but with only one chromatid of each chromosome labelled in the euchromatin regions. 
- Same plate after deparaffinization. 
- A ch centphase with labelling mainly on the heterochromatin regions of all chromosomes. 
- Another ch centphase with persistent labelling of heterochromatin regions but in some monosomes, one of the chromatids is distinctly unlabelled (→), x 2000.
replicating modal in brain ganglia are being synchronized; however, the mitotic chromosomes in these 24th M18-20a larvae show some damage and therefore, in brain experiments shorter duration of 5th treatment was applied. It was found that a 24 M18-feeding also induces as good synchrony in replicating brain nuclei as a 48th feeding period but with little chromosome damage and therefore, in the experiments reported here, all brain larval larvae of 24th were fed an M18-feeding for 7h only. After this synchronization period, the brain ganglia were picked and one set was immediately post labeled with 3H-thymidine (Table 6A) for 30 min and fixed. A 2nd set of ganglia was cultured in vitro for 24h in the continuous presence of 3H-thymidine (0.001µc/ml) and unlabeled (0.5µg/ml). In a 3rd set, the excised ganglia were fixed labeled with 3H-thymidine for 15% which immediately was used in the assay medium and 5h later the ganglia were harvested.

In each of the above cases, after the period of labeling air-dried chromosomes preparations were made and autoradiography in the usual manner. For each labeling scheme at least 8 brain ganglia were used. The availability of 3H-thymidine in the medium after the 24h of labeling period was assayed by culturing fresh ganglia in the used culture medium; in each case it was found that the fresh ganglia are as well labeled in these medium as in the fresh medium and this assures that the label was present in the matrix for the waiting duration.

The autoradiograms were scored for the pattern of labeling on interphase and metaphase nuclei. The data on the labeling pattern of interphase nuclei in 24 pulse and 24 hour cultured metaphase during the last 3h labeled ganglia from unimmunopurified and 10 µg 3H-thymidine-labeled larvae are presented in Table 1. In all cases, no 24th interphase nuclei (larger as well as smaller sized) remained unlabeled while among the labeled nuclei, 3 categories are seen i.e., the interchromosomal chromatographic labeled; (ii) the extrachromosomal non-chromatographic labeled; and (iii) both regions labeled (fig. 2). It is seen that compared to the 24 pulse labeled ganglia, the frequency of completely labeled nuclei increases while those of chromatid labeled nuclei decreases in 24h duration labeled ganglia both in the unimmunopurified and immunopurified series (see Table 1). This shift is more pronounced in the 3H-thymidine-purified ganglia since it is the initial frequency of extrachromatin labeled nuclei to reach higher level which indicated to us in the case of ultraviolet light and x-irradiation treatment of tissue; the labeled nuclei are blocked replicating nuclei at the beginning of their repair in the case of ultraviolet light and x-irradiation treatment and blocked replicating nuclei at the beginning of their repair in the case of ultraviolet light and x-irradiation treatment. The treatment of brain tissue (0.5µg/ml) is significant and appears to be related to the earlier discussed amount of asynchronous cycles of grow and hypochromatin region of a neuron prior to 24h. These nuclei may have been programmed or engaged in desynchronization of hypothecrosis and upon release from the block by 3H-thymidine, they remained asynchron in hypochromatin, 24h immunopurified as well as we synchronized ganglia labeled with 3H-thymidine for 24h, about 50% labeled still have interchromatin labeled (Table 2). In an earlier study (0.5µg/ml) we found about 30% showing 3H-thymidine only in the hypochromatin region during a 48h long labeling period. A consideration of the labeling patterns of
For analyzing the replication patterns of cells growing into discrete, long periods of labeling (14 and 28) have been used and also similar periods either given for the entire period or only during the last 5th of labeling. In the case of typical cyclically cycling cell populations in which during every 5th period, subchro-
netic replication is early and late-5 is chronologically followed by heterochromatin replication in late 5, specific predictions can be made regarding the labeling patterns observable on metaphases in such experiments. In an asynchronous proliferating population of cells labeled for long periods (longer than average cell cycle duration) with tritiated thymidine in the presence of colchicine, the proportion of unlabelled and only heterochromatin labeled metapho-
As should decrease with increasing duration of labeling and cell-division exposure time during the longer, blue and red, and more white, black and blue labels would have traversed through the interphase and got blocked when growing metaphase stage, then covalently labeled metaphase slice would consistently ablate the pool of covalently labeled metaphase slices. On the other hand, in these chronologically labeled metaphase slices, the proportion of unlabeled and heterochromatin labeled metaphase should be very low if the average cell cycle duration is shorter than the metaphase labeling period. It may be that the 26th metaphase labeling period employed in this study is longer than the telophase of the average cell cycle duration in brain of quails of *Troglodytes aedon* (17.2 days).

Results of the present study, shown in table 3, do not confirm the above expectations. Before considering the results, it may also be noted that under the in vitro culture conditions used in these studies, the proportion of cells in mitosis is not disturbed since the mitotic index has been shown to be progressively higher with increasing length of colchicine exposure (data not presented, but see fig. 3). Besides the frequency of 4n or even 2n metaphase (arising due to progression of one of the arrested metaphase to 2nd or 3rd mitotic cycle) has also been found to increase with increasing colchicine exposure periods. Thus the present results are not distorted due to impaired mitotic progression rates. To examine the observed labeling patterns some ideas of the 1st cycle metaphase, the 4n metaphase present in different samples have not been included in the data presented in table 3. In the synchronized and 26th labeled samples, although the frequency of unlabelled metaphases in quails exposed for mitosis only for the last 5h is nearly half that in the 26th colchicin exposed samples, the frequency of heterochromatin labeled metaphases in only slightly less in the 5h population (31.7%) than in the 26th metaphase (39.5%) sample (see Table 3). The higher frequency (46%) of colchicine labeled (unlabelled metaphases in quails labeled with H-thymidine for 4h in the presence of colchicine) is also contrary to the expectations based upon typical mitotic cell cycles, due to the length of mitotic cell cycles in these quails, the mitotic cells have been distinguished into 26th synchronized stages compared to those in the uncolchicinized cultures exposed to colchicine and the uncolchicinized cultures exposed to 26th synchronized stages. However, in these 26th metaphase labeled synchronized quails, the proportion of heterochromatin labeled metaphases in the 26th population exposure varies (16.6%) is not much reduced compared to that in the 5h colchicine exposure series (12.2%), table 3.

These observations on the metaphase labeling patterns have confirmed in conjunction with the earlier (20) demonstrated absence of hetero- and euchromatin underreplication cycles in larval brain cells and the interphase labeling data presented here, lead us to believe that in the larval brain tissue a cell entering replicating phase may have replicated only the heterochromatin regions during the subsequent replication period, considered to be significant only during the earlier endoreplication phases, the heterochromatin region of a nucleus replicated a few times more than the euchromatin although that the quails at this stage saw a disproportionately large heterochromatin content prior to endoreplication cycle. Thus this cell
how to water stimuli, it may need to replicate only its heterochro-
matic regions. In primate studies (Broca), evidence was obtained
for frequent replication of the heterochromatid regions during
unipolarization cycles in early stages of viral development.

Since brain cells which have had replicated acro- and heterochromatic
regions in independent unipolarization cycles have been seen to
water stimuli with multi-replicated chromosomes, the possibility that
all in primate chromosomes, in the polyene metaphase chromosomes
also the number of lateral strands in the acro- and heterochromatin
regions is unique, needs consideration. This situation would arise if
unreplicated cells lacking inappropriate levels of hetero-
- and euchromatin were to be triggered to enter mitosis. Evidence in
favor of these events have been obtained in the results of above
mentioned experiments. As mentioned earlier, in the preparations
of brain ganglia labelled simultaneously with BrdU by 20 or 40
in the presence of colcemide, 60 metaphases are also present.
In the unreplicated set, about 60 of all metaphases in the 20
sample and about 1/4 in the 40 sample are tetraploid. In the syn-
chrotron 24th set, the frequency of all metaphases is higher (70)
presumably because of the faster induced synchronic progression of
(i.e., 1) of all the metaphases are labelled with BrdU. However, it is
very significant that at least 10 to 1/4 of these BrdU labelled show
labelling of only the heterochromatic regions (fig. 4). Since these metaphases are never seen after a
shorter colcemide exposure (2,7), these appear after the longer
colcemide treatment must be the arrested 28 metaphase cells enter-
ing a second mitotic cycle. Thus all the 80 metaphases cells seen
in this study must have traversed through at least one S-period in
the presence of BrdU. Usually, these BrdU metaphases then
should show uniform labelling of all as well as heterochromatin
regions. Labelling of only the heterochromatin regions in some of
the BrdU cells shows that the euchromatin regions of different chro-
mosomes did not replicate in the S-period immediately preceding the
phase. This implies that either those 60 metaphase have unde-
replicated heterochromatin or that at the 20 stage, the metaphase
chromosomes contained non-replicated heterochromatic regions. In
another case, these results make it possible that metaphase chromo-
sones with unequal number of lateral strands in hetero- and euchro-
matin regions may occur in larval brain ganglia. Whether the comple-
tely labelled metaphases are also have unequal lateral multiplicity
of certain regions can not be established from present results. We
are now examining this aspect by cytophotometric methods.

Results comparable to the above have also been obtained in parial
experiments (data not presented here) using bromodeoxyuridine (Br-
dU)-heterochromatin incorporation andlines staining and autoradi-
ography studies.

Other unusual types of autoradiographic labelling have also been
seen in the 28 and 40 metaphase in these preparations of brain
ganglia chronically labeled with BrdU for 0 or 40.
In all samples, in 10 to 50% of the heterochromatin regions in these chromosomes were not labelled. In a few of the 28 metaphase,
both chromosomes of all chromosomes were labelled. In hetero-
chromatin regions, in some of all the metaphase chromosomes present
only on one of the two sister chromosomes of all or some
chromosomes (see fig. 4). Likewise, in several other 28 metaphase,
While all sister centromeric regions were labeled, several inter- 
centromeric segments were not labeled. Significance of such label- 
ing patterns is not immediately clear but their presence is not 
indicative of either unusual patterns of replication or segrega- 
tion of the label to daughter chromosomes in these polychromat- 
hya.
to ana- or mitotic replication cycles. Moreover, pulse-labeling studies alone do not provide information about the endoreplicating cells and their progression to mitosis. Thus Stielstra et al. [9] results are not necessarily in conflict with the present observations, although these interpretations in that study appear to be too simplistic and based on incorrect assumptions of larval brain cells being diploid and exponentially progressing.

To summarize the above, the following proposal in made to satisfactorily explain the varied observations made on the replication patterns in brain cells of *Drosophila* larvae. In these cells the endoreplication and mitotic cycles are not mutually exclusive; a transition probability is assigned to the transition probability of entry of G1 or G2 cells into S-phase in other mitotically cycling cells, see ref. 16. Severeams the presence of an endoreplicating cell to mitotic division cycle with polyteny chromosomes, thus an endoreplicated cell may quickly divide after its last DNA synthesis period or may remain quiescent for a longer time before it is triggered to enter into a polytenic division. During the intermitotic period the nucleus may endoreplicate its own and heterochromatin regions in independent and possibly unequal cycles.

D. CURATIVE AND DEVELOPMENTAL IMPLICATIONS OF UNIPOLARITY DURING EMBRYONIC CELL CYCLES

Role of heterochromatin in cellular activity is not known. Yet the quantity and quality of heterochromatin in the genome is well known to influence the phenotype in various ways in *Drosophila* and other organisms. It is also significant that the satellite sequence repopulation in different cell types of *Drosophila* larvae and adult varies in a characteristic manner (3). Apparently, this variability is generated by specific patterns of replication.

 Unequal participation of different DNA sequences in polyteny replicated cycles in *Drosophila* has been demonstrated in several cases and stipulated in others (see Section B). Our results (Section C) on the larval brain cells have raised the possibility of polyteny unequal replication of different sequences in mitotic chromosomes as well. In the simplest terms, we may presume that the same euchromatin molecules cause and regulate differential replication of specific sequences in polytene chromosomes, are opposite in mitotically active polytenic artisan cells alone. It is very likely that a multistranded organization of mitotic nuclei provides the basis for gene regulation and propagation of cells having different relative amounts of satellite and other sequences in a cell and tissue-specific manner. Non-polytenic cells in adult tissues of *Drosophila* have been found to harbor varying amounts of different satellite sequences (1). However, Spradling and Hauskeil (23) have shown amplification of certain genes in polytenic cells in an adult *Drosophila*. This latter observation also raises the possibility that mitotic genome replication of highly and polytenically enlarged cells of *Drosophila* may be more common than hitherto believed (24). It is significant that the so far demonstrated instances of selective gene amplification in *Drosophila* cells have been found either in highly polytenic cells (RNA puff in *Drosophila*, refs. 19) or in endoreplicated ovarian follicle cells of *Drosophila* (30) the possible unequal polytenization of different bands in salivary glands of *Drosophila* larvae (14,40) may also be related to macronuclear gene amplification processes. Therefore, it may appear that endoreplication cycles
are associated with the presence of selective gene amplification, at least in *Drosophila*. Our demonstration of widespread occurrence of endoreplication cycles even in the normally entire somatic cells of *Drosophila* thus lends significant from the viewpoint of cell differentiation.

An interesting little understood process related to heterochromatin is the phenomenon of position-effect variegation. In *Drosophila* and several other organisms, it is known that when a microchromosomal rearrangement relocates a euchromatic region in or very close to heterochromatin, the expression of genes in the rearranged position becomes variable and this results in a variegated phenotype. If the rearranged segment has a dominant wild type gene and the unaltered homolog carries the same recessive allele, extensive genetic and developmental studies have been carried out to ascertain effect variegation in *Drosophila* and these have been reviewed several times recently to which reference may be made for details ([11], [40]). However, despite these extensive studies the basis for the variegated expression of genes transposed close to heterochromatin is not understood. Nevertheless, it is generally presumed that the activity of the euchromatic locus when placed in proximity to heterochromatin is inhibited in some but not all cells and this results in the variegated expression. Evidence shows that the variegated gene is not physically lost from the cells showing mutant phenotype but its activity is altered ([11]). In important features of variegation is its sexual expression; i.e., in a clone all cells will either have the rearranged gene in active state or active either, moreover, the extent of activity may vary in the transposed locus in male only before the gene has to actually express itself. This same a limitation for inactivation of the rearranged locus has been made in a cell, all its progeny continue to show inactivity of the locus (see ref. cited for further details).

Due to improved cytogenetic resolution offered by polytene chromosome, a Cytogenetic Approach has also been made to study this phenomenon. Without going into details of the various observations on this aspect, it may be briefly stated that the euchromatic segments transposed to heterochromatin generally show a variable degree of union-replication and heterochromatization ([10], [11]), the altered nucleolus and expression of polytene ([40]) of variable amount-replication of euchromatic slow transposed to heterochromatin as seen in polytene nuclei may provide a basis for the variegated phenotype with the suppression of heterochromatization of the rearranged locus, its transcriptional activity may be more or less ([40]). However, one recent study by Brown ([50]) has led to a hypothesis derived from in a variegating locus in polytene nuclei and has found that the locus of insertion in a variegated state is greatly affected at the inactive gene region and thus has implied that the variable ability of a gene to be transcriptionally induced is dependent upon its gene dosage. However, we also noted by Brown ([50]) in which heterochromatin would not diminish the different types of polytene as previously in cytoblastomycosis. In this context, another observation by Overell and Hebermann ([40]) is interested. They found by microchromosome of polytene nuclei that while the transposed euchromatin in an immeasurably fast to heterochromatin in a variegating condition contains more than the normal euchromatin, an active
cent intermediary heterochromatin will on the transposed chromosome has over than the homologous site on the normal chromosomes. We have also observed (J. Mihara and Takahashi, unpublished) that in polystyrene nuclei of /brachymetamerus larvae carrying mdr inversion in the X-chromosomes (see details of the inversion, see ref. 21) the replication program of certain bands away from the heterochromatin relocation point is altered in a characteristic manner although some bands closer to the breakpoint are not so affected. Viewed in the light of these results, Hamada's (20) data do not eliminate the possibility that while the replicative program of the coding sequence has resulted relatively unchanged in this material, that of certain other neighboring sequences does get modified in the transformed chromosomes and which in turn may influence the instability of replication at the locus in the transposed chromosomes. Taken together these observations in salivary gland polytene chromosomes generally support the view held by Schatz (42,45) that the variated expression of a relocated gene is related to the altered endoreduplication program. Still, it has generally been implied that the altered endoreplication programs of a relocated gene may be a phenomenon restricted to non-proliferating polytene-like nuclei. The proliferating anterolar cells, whose terminal progeny contributed to the bulk of adult Brachyplus body, have been believed to be diploid and therefore, alterations of the post-synapsis polytene replication could not be visualized to be the basis for position effect variegation in imaginal cells (see ref. 47). However, the view proposed here that structurally proliferating cell in Brachyplus may also endoreplicate makes it possible to think of a unified basis for position effect variegation in all cell types of Brachyplus. In another study (52) unidentifiable wing imaginal disc cells in the uniform larvae of Brachyplus also have been seen to be engaged in endoreplication cycles with disproportionately replication of hetero- and euchromatin. In a more recent study (53) we have obtained evidence for such endoreplication cycles in wing imaginal discs even during early larval stages. Revisiting the results obtained with larval brain nuclei, it would appear that the situs chromosomes in imaginal disc cells are also polytene.

In view of the above discussion, the following proposal is made in relation to polytene effect variegation in Brachyplus. Anterolar cells of Brachyplus endoreplication cycles occur with or without intervention of other factors. After the endoreplication cycles, various levels of under- or over-replication of specific sequences occurs in a tissue-specific manner. In instances of position effect variegation, these replication cycles are variably modified under the influence of neighboring sequences and this leads to the replication expression of the affected gene in different cells homologues of differences in the quantity and quality of the coding sequences or of other associated sequences or both. The interpretation of situs disorders with endoreplication cycles makes it possible that a particular level of under-or over-replication of certain sequences can be clonally propagated. This proposal satisfactorily expounds the genetic initiation of the variegated effect. The modifying effects of extra heterochromatin, developmental, temperature and other factors on the extent of variegation (42,45) may operate through modulation of the endoreplication cycles.
Recent research of Cren and Rana (11) on the altered levels of 5-HIAA sequences in brain ganglia of *Drosophila* larvae carrying certain *P-element* translocations may be relevant to position effect variegation such that caused by the degree of promoter specific variegation of 5-HIAA sequences in the adult male *Drosophila* larvae, the specific *P-element* translocation causes the 5-HIAA sequences to escape under-repression during the embryogenesis cycle. Earlier observations by Meyer (14) on the altered levels of 5-HIAA content in certain *P-element* translocations of *Drosophila* which relate the molecular organization regions from its central heterochromatin site to the vicinity of euchromatin may also be related to the changed programming of replication sequences of 5-HIAA in the transformed position.

If the above proposed mechanism of position effect variegation in *Drosophila* is valid in its essential principles, the molecular basis of the similar phenomenon associated with the mating *Drosophila* males in female hemizygous (16) would be basically different. The apparent similarity in the two systems of variegation in *Drosophila* and maize may not imply a common causal cause. While in *Drosophila* this phenomenon is associated with facultatively heterochromatized (15) *D* chromatin in *Drosophila* and several other organisms this phenomenon is related to de novo genotypes involving constitutive heterochromatin. The activation of *D* in male *Drosophila* males has been suggested to be due to specific patterns of methylation of cytosine (17) while in *Drosophila* genomic methylation of is also true (4, Biggs, personal communication) and thus the "spreading" of methylation to the released euchromatic segments may be due to different causes in the two instances. It remains to be seen if chromosomal rearrangements involving constitutive heterochromatin in maize also cause position effect variegation.

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