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Nature of Heterochromatin*

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I. Introduction

RECENT developments in molecular cytogenetics have revived interest in the nature and function of heterochromatin. The concept of heterochromatin, from its very inception, has been one of uncertainty and vagueness. The data obtained from various cytological and genetical sources have not in any way helped in developing a unified concept of heterochromatin. The literature is exhaustive, both with respect to direct observations and theoretical speculations. In 1950's, though the importance of heterochromatin was realized, there was, in general, a resigned approach to the illusiveness of heterochromatin[1-3], and this was summed up by Pontecorvo[4] in the following words: "the study of heterochromatin is at a prescientific level ..... We have no alternative but to ignore it." However, heterochromatin was never ignored, and the 60's saw a renewed interest, and the various aspects of heterochromatin have been analysed by ever-increasing number of workers with new techniques. These studies have now provided a new insight into the nature and function of heterochromatin. The functional aspects of heterochromatin have been recently surveyed by Yunis and Yasminch[5]. In the present review several aspects of heterochromatin, particularly the recent developments, have been considered, and an attempt has been made to analyse the unity in diversity of the nature of heterochromatin.

II. Development of the Concept of Heterochromatin

The original concept of heterochromatin was purely cytological, when Heitz[6] observed differential condensation of some chromosome segments at telophase in the liverwort, *Pellia*. Such segments were termed 'heterochromatin', by analogy with the 'heterochromosomes', the name coined by Montgomery[7] for 'heteropycnotic'[8] sex-chromosomes of many insects. Heitz[6] called the other chromosome regions 'euchromatic', which showed a dispersed state at telophase and interphase as compared to heterochromatin, which remains condensed.

This cytological entity was soon endowed with genetic characteristics. Much of the earlier work on *Drosophila* showed genetic inertness of heterochromatin[9-13]. Stern[14] had shown earlier that the Y-chromosome (entirely heterochromatic) in *Drosophila* was essential for sperm motility, though without any apparent effect on the viability or the sex-phenotype of the male, as even in the absence of a Y-chromosome, the flies were normally viable and of male phenotype[15].

The original concept of total inactivity of heterochromatin could not be maintained for long, though the idea of absence of major genes on heterochromatin is held to date. Gradually, a confusing array of 'functions' of heterochromatin were proposed. Mather[16] suggested the localization of 'polygenes' for quantitative traits in the heterochromatic regions. Position-effect variegation was also found to be associated with heterochromatin[17-20].

A new dimension to the study of heterochromatin was added by tritium-autoradiography[21]. Lima-de-Faria[22] first showed that in grasshopper and rye, the heterochromatin synthesizes its DNA later than euchromatin. Taylor[23] confirmed this result in mammals, and since then, exhaustive studies on replication in a wide variety of materials have been made, and the results have strengthened the correlation of 'late-replication' with heterochromatin[24-26].

The confusion about heterochromatin was clarified to some extent by Brown[27], when he proposed the terms '*facultative*' and '*constitutive*' heterochromatin for two basically different kinds of condensed or inactive chromatin. 'Facultative' or 'functional' heterochromatin involves only one homologue, while the other remains active and euchromatic in the same cell. The 'constitutive' or 'structural' heterochromatin is believed to involve both the homologues at the same time in one cell in condensation and inactivity[27]. The last decade has seen phenomenal increase in our understanding of the nature of heterochromatin mainly due to the works in two systems, viz. the mealy bugs[27,28] and the mammalian chromosomes[5,29-34]. The recent developments in the techniques of *in situ* hybridization[35,36], the specific Giemsa-staining[37,38] and the acridine-dye binding fluorescence[39,40] have provided significant information about the architectural and functional role of heterochromatin in cellular dynamics[5].

III. Properties of Heterochromatin

During cytogenetical studies, heterochromatin has been characterized in various ways and by different properties. These properties of heterochromatin are briefly discussed below (Fig. 1).

(1) *Condensation and heteropycnosis* — Condensation, allocycly and heteropycnosis are the most easily observed manifestations of heterochromatin. It appears to be the fundamental property of heterochromatin to remain condensed when the rest of the chromatin is in a dispersed state. Thus, in interphase, heterochromatic regions may form condensed and darkly stained bodies or chromocentres and frequently these chromosome regions also show allocycly even at metaphase, i.e. remain more condensed and darkly stained than the euchromatic regions of chromosomes (Figs. 2 and 3). In the

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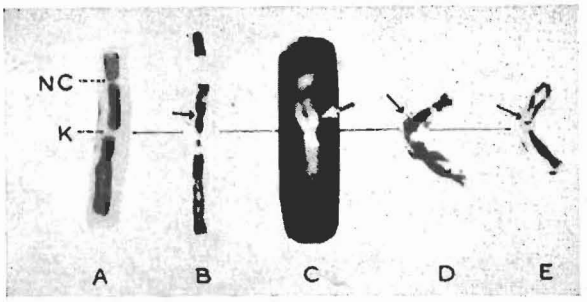

Fig. 1 — Properties of heterochromatin segment in M-chromosome of *Vicia faba* (A) Localization of heterochromatic segments; K, kinetochore, NC, nucleolar constriction; (B) effect of cold (6°C for 72 hr), heterochromatic regions less condensed; (C) quinacrine mustard binding segments; (D) late-replicating segments; (E) chromatid break in M_1 heterochromatic segment induced by mitomycin C. Note the coincidence in the chromosomal segment M_1 (arrow) in all the cases[92]

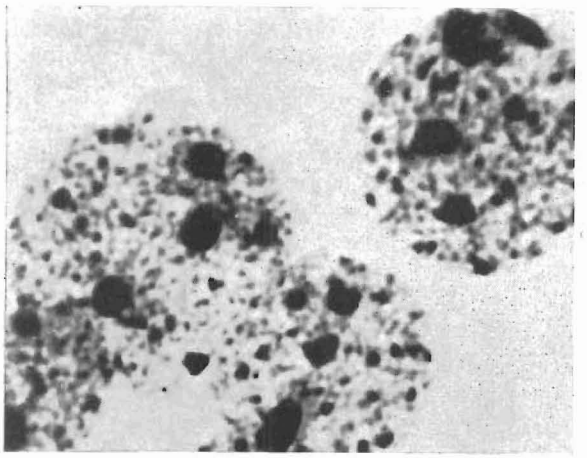

Fig. 2 — Heterochromatic chromocentres in liver interphase nuclei of *Rattus blan fordi* (Carbol-fuchsin stain)

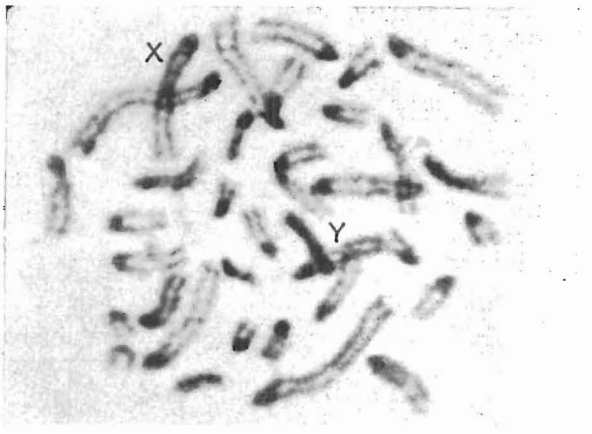

Fig. 3 — Heterochromatic segments in metaphase chromosomes (bone marrow) of male *Rattus bland fordi* [Note the darkly stained centromeric regions in all chromosomes and the proximal half of X and entire Y-chromosome (Carbol-fuchsin stain)]

heterochromatic regions, the DNA content per unit area is greater than in euchromatin[22], which might explain the normal condensed state and deep staining of such regions.

In some cases, there is negative heteropycnosis[41], *i.e.* some chromosome regions appear more diffuse and light-stained at metaphase. Lima-de-Faria[26] has emphasized that all such negatively-heteropycnotic regions should not be considered heterochromatic. However, some heterochromatic regions do show negative heteropycnosis at metaphase, especially after some treatments like cold, colchicine, etc.[42-45] (Fig. 1). It is, of course, true that such non-staining gaps are not due to the so-called nucleic acid starvation[46], but are probably due to some differential coiling of the chromatin. In an elegant study on the heterochromatin of the Indian Muntjac, Comings[45] has clearly shown that the differential response of heterochromatin to colcemid is due to differential contraction. In this case, euchromatin contracts more than heterochromatin. It has also been shown that this phenomenon is unrelated to DNA synthesis[45].

(2) *Genetic inactivity and inertness* — It is widely believed that heterochromatin is genetically inactive. At times, it has also been implied that heterochromatin, being genetically inert and 'empty', may be dispensable[47,48].

Studies on *Drosophila melanogaster* have shown that in this species, the entire Y and the proximal one-thrid of the X-chromosome are entirely heterochromatic and devoid of the so-called 'Mendelian' genes as well as the sex-determining factors[9,10,14,49,50]. In all the other systems analysed, it has been observed that heterochromatinization is associated with either the absence or the repression of genetic

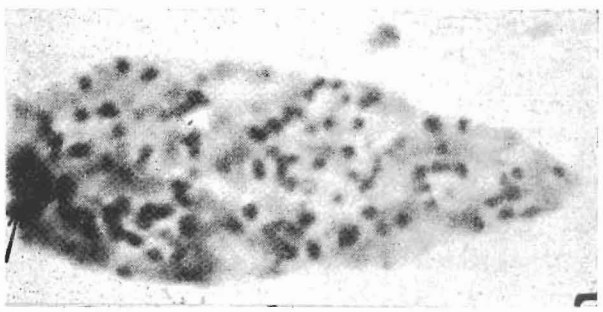

Fig. 4 — Absence of ^{3}H-uridine incorporation in the sex-chromatin in *Funambulus pennanti*[53]

Fig. 5 — Late-replicating 'Lyonized'-X chromosome (arrow in *Suncus murinus*

activity[5,26-29,31,51,52]. This is also manifested at the molecular level by absence of transcription[51,53-55] (Fig. 4). Genetic inactivity and inertness of heterochromatin is discussed in later sections; it may be mentioned here that heterochromatin or condensed chromatin is believed to be not manifestly active in transcription.

(3) *'Late' replication*—The first report on the asynchronous replication in heterochromatin and euchromatin was by Lima-de-Faria[22]. The heterochromatic X-chromosome in the germ cells of *Melanoplus* was found to complete DNA synthesis later than euchromatin[22]. Since then this property of heterochromatin has been analysed in very different systems, and barring a few exceptions, in all cases, heterochromatin has been observed to be 'late' replicating (Figs. 1 and 5). Recently, the relationship between condensed chromatin and 'late' replication has been analysed in detail by Lima-de-Faria[25,26], and it could be concluded that heterochromatin completes replication later than euchromatin. As also stressed by Lima-de-Faria[26], the DNA replication is affected by differentiation, and as such, a change from heterochromatin to euchromatin or vice versa may also be associated with a change in replication timings[56].

Another characteristic of heterochromatin replication is the late beginning of DNA synthesis[26]; however, the asynchrony in the beginning may not be as pronounced as in the terminal period and in some cases no asynchrony could be detected. Thus, in *Drosophila* polytene chromosomes, the euchromatic and heterochromatic regions start DNA synthesis at the same time, but the heterochromatic regions complete it later than euchromatin[57-59]. In *Chironomus* polytene chromosomes, however, the heterochromatic bands both start and finish DNA synthesis later than euchromation[60].

The rate of replication is also differential in heterochromatin and euchromatin. Several studies in mammalian chromosomes have shown that heterochromatin takes lesser time to complete DNA synthesis than the corresponding euchromatin[45,61-64]. In a recent study on the heterochromatin replication in hedgehogs, it has been suggested that heterochromatin is replicating twice as fast as euchromatin[64]. More replicons are believed to be replicating in synchrony in heterochromatin[45,63,64]. However, this replicative behaviour may not be universally true for all the heterochromatic regions. Obviously, in cases where both heterochromatin and euchromatin initiate DNA synthesis at the same time, but the former finishes late, the rate of DNA synthesis in heterochromatin may not be higher than in euchromatin. However, in such cases, the amount of DNA and its organization in the two kinds of chromatin have also to be taken into consideration. Further studies on the kinetics of replication in euchromatin and heterochromatin in different systems are needed for a clear understanding of the replicative organization of the various chromatin fractions.

It must be emphasized, however, that all 'late' replicating chromosome regions need not necessarily be heterochromatic. In a nucleus, there are many replicating units[59,63,65-59], and these different units complete their DNA synthesis independently in the S-period, depending on the amount and organization of DNA[32,59,69,70]. Thus, some replicating units in a nucleus will be finishing their DNA synthesis when others have already completed. It is not imperative that all such late-finishing replicating units be heterochromatic, though the latter would be represented in such late-replicating units.

Late-replication and condensed state of heterochromatin are probably interdependent. Several studies have indicated that the heterochromatin or the condensed chromatin undergo decondensation during DNA synthesis for a brief period[71-73]. This decondensation may be a prerequisite for replication. Kuroiwa[74] has also shown by electron microscopic autoradiography that the late-replicating or the heterochromatic regions condense faster than euchromatin during prophase. Thus, it seems that cellular regulatory systems control the condensation cycle of different chromatin materials in such a way that heterochromatin is decondensed for only a brief interval during the S-period, and this may also be related to the genetic inactivity or the repressed state of heterochromatin.

(4) *Nonhomologous or ectopic pairing*—A feature of heterochromatin frequently referred to is the 'stickiness' or the association of nonhomologous regions at interphase. The common chromocentres formed at interphase are the results of such associations[75-77]. This is best seen in polytene nuclei. In *Drosophila*, the centromeric heterochromatin of different chromosomes fuse to form one common chromocentre, while the intercalary heterochromatin, distributed throughout the length of the chromosomes, shows 'ectopic' pairing, or thread-like DNA containing interconnections between two nonhomologous loci. Several studies[78-81] have revealed that the ectopic pairing sites show all the characteristic features of heterochromatin, namely a condensed state, high susceptibility to induced breaks, intense fluorescence and late-replication[57,79-83]. Ectopic pairings may occur by accidental sticking of the projecting loops into nearby chromatin, and this may be an indication of redundancy of DNA or certain other common properties of deoxynucleoprotein in heterochromatin. It is interesting to note that facultative heterochromatin[27] often fails to form such common chromocentres[77-84].

(5) *Susceptibility to induced breakages*—Various factors are known to induce chromosome breaks[85]. Heterochromatic regions have been found to be especially susceptible to such induced aberrations in both animal and plant cells[77,85-94].

Mitomycin C has been used frequently to induce chromosome aberrations and as recombinogen in higher organisms[85-98]. In a study on the action of mitomycin C on the chromosome aberrations in *Vicia faba*, a correlation between breaks and the late replication of heterochromatin has been demonstrated[92]. After *in vivo* synchronization of the cells in the root tip of *Vicia faba*, the aberrations induced by mitomycin C were maximum during the late S, and of all the scrorable breaks, 93·5% were in heterochromatin, indicating that the breaks are induced when the chromosomes are replicating[92,93]. A similar explanation has been put forward by Haegle[94] for FUdR induced breaks in the polytene chromosomes of *Chironomus*. Heterochromatic regions also show a high frequency of induced somatic crossing over[90,91,95,99-102]. It is possible that these two aspects of heterochromatin are manifestations of the same basic organization.

During interphase, the heterochromatic regions remain condensed, and often different, such regions associate into larger blocks of chromocentres (cf Section III, 1 and 3), and this provides a basis for 'misrepair' of the induced scissions in the DNP fibrils, leading to chromosomal aberrations or crossing over. Late-replication may also favour 'misrepair'[77].

(6) *Position-effect variegation* — A variegated expression of a gene due to its new position near heterochromatin is now regarded as a generality of variegation and heterochromatin[20]. Position-effect variegation is seen when genes normally located in euchromatin are brought within or in close proximity of a broken heterochromatic region; a *cis*-arrangement is essential for variegation[20]. Most of the earlier studies were on *D. melanogaster*[19] and later on extensive studies have been made on *D. virilis* and mouse[20,33]. Studies in mouse on the position-effect variegation have been particularly rewarding, since in mammals, as discussed later, one of the X's in female is heterochromatinized in somatic cells. When there is an autosome-X translocation or insertion, the autosomal genes often show variegation[33].

The basis of variegation in these instances lies in the inactivation of the transposed and normally active gene due to its new proximity to the heterochromatin. The 'spreading-effect' associated with position-effect variegation is also very interesting and important. There is a polarized gradient of inactivity emanating from the broken heterochromatic region, and this affects the newly transposed euchromatic regions. It is the variablity of the 'spreading-effect' that produces variegation. The inactivity or lack of realization of gene products of these newly transposed regions is still explained in terms of heterochromatinization or compaction. This, as pointed out by Baker[20], "in reality, exposes our ignorance rather than our understanding". Recently, Eicher[33] has proposed an attractive mechanism for heterochromatinization, and variegation in particular; this model can also be extended to gene regulation in general. On the basis of this model, each gene is believed to have a starter and a terminator, and heterochromatic regions are believed to be a group of polarized starters and terminators working as a unit under the influence of a common receptor site. Thus, a new gene, brought near heterochromatin due to breaks and rearrangement, too may become inactivated under the polarized influence of the 'receptor-starter-terminator' complex[33].

Whatever be the mechanism of inactivation, the phenomenon of V-type position effect is a clear indication of the supragenic level of organization of chromosomal activity and a hierarchy of levels of regulation. It is to be realized that the concept of supragenic levels of control is fundamental to an understanding of heterochromatin. The various properties of heterochromatin, like condensation, 'late'-replication, 'inactivity', position-effect variegation, etc., are not independent responses of the individual units located in these regions, but they probably are collectively controlled by a 'master' regulator (*see* also Section VI).

Recently, Baker[103] has provided evidence for a reverse type of position-effect on the r-DNA cistrons in *D. melanogaster*. He could demonstrate that the r-DNA cistrons, normally located in the X- and Y-chromosome heterochromatin, show a position-effect variegation, when placed in the euchromatic regions, due to a break between these cistrons and the centromere[103]. This also suggests that there is a specific organization and regulatory action within the normally heterochromatic regions, and this may be disturbed when such genes are removed from their influence. It is interesting to note that heterochromatin shows other types of gradient effects also, e.g. in the differential contraction following exposure to cold or colcemid. Thus, in a study on the heterochromatin in Indian Muntjac, Comings[45] observed that "there is a gradient of chromosome contraction with the least contraction occurring in heterochromatic centromere regions and the greatest contraction occurring in segments that are farthest removed from the centromere". The similarity in the gradient effects in position-effect variegation and differential contraction is striking, but it is not known whether these two aspects are related.

IV. Facultative and Constitutive Heterochromatin

Heterochromatin is now usually grouped into two broad categories: facultative and constitutive. This concept, introduced originally by Brown[27], has provided a basis for reasonable grouping of a wide, and often confusing, array of instances of inactive or condensed chromatin. Earlier, all such cases were labelled simply as heterochromatin, and perhaps, this is one reason why so much ambiguity about the nature of heterochromatin has come to stay. Initially, Heitz[104] classified *Drosophila* polytene chromosome heterochromatin into α- and β-heterochromatin on the basis of their ability to uncoil. Though this classification is still meaningful (*see* Section IV 2.C), this grouping does not provide easily operative criteria and has not been much popular. On the other hand, the concept of constitutive and facultative heterochromatin has found wide acceptance and usage.

Brown[27] proposed that chromosomal regions which show heterochromatinization in one homologue, but remain euchromatic in the other, should be termed 'facultative' or 'functional' heterochromatin. The regions showing heterochromatinization in both the homologues in the same cell are termed 'constitutive' or 'structural' heterochromatin.

1. *Facultative Heterochromatin*

Best known examples of facultative heterochromatin are the 'inactive' or the 'Lyonized-X' in female mammals[105–107] and the paternal set of chromosomes in diploid male mealy bugs[27,28,108].

(A) *Mammalian X-chromosome* — In all female mammals, one of the two X's in somatic cells becomes inactivated and heterochromatinized and continues to remain so in subsequent cell generations[105,106,109], and this expresses itself in interphase as the sex-chromatin or 'Barr' body[110]. At metaphase, this inactive-X can be seen as the 'late'-replicating or 'hot'-X in female cells[23,24,26,32,53,111–115] (Fig. 5). This condensed and 'late'-labelled X-chromosome in female mammals has been elegantly and extensively correlated to genetic inactivity at morphological, biochemical and molecular levels[29–34]. It has been shown that the sex-chromatin is inactive in RNA synthesis[53,54]; recently, some workers[116,117]

have, on the other hand, suggested that the 'inactive'-X synthesizes a small but significant amount of RNA. However, this needs confirmation using different experimental procedures. It is nevertheless possible that the 'Lyonized'-X may not be completely inactive[33,34,118,119]. In general, however, one of the X's in female mammal is randomly inactivated and heterochromatinized in early embryogenesis somewhere before implantation[34,120-123], while the other X-chromosome remains functional and euchromatic. In males, there is no heterochromatinization of the X in somatic cells. This differential behaviour of the X-chromosome in male and female, and also the differential activity of the two X's in the female is illustrative of the nature of facultative heterochromatin.

(B) *Coccids* — In the mealy bug group of coccids, the males show a typical example of facultative heterochromatin. In all males, the haploid set derived from paternal source is heterochromatinized and its activity is repressed. These heterochromatic chromosomes are retained in the cells of somatic tissues. Extensive studies[27,28,51,108,124,125] have shown the genetic inactivity of these condensed chromosomes, which are also late replicating[126]. The haploid set of maternal origin retains its functional state and euchromatic nature. The interesting aspect of this heterochromatinization is the reversibility of the heterochromatic paternal chromosomes to euchromatic state in some organs[28,125,127,128].

(C) *Nature of facultative heterochromatin* — Observations on facultative heterochromatin indicate that this category of condensed and inactive chromatin is not a *distinct kind* of chromatin, but is only a *state* of differentiation. It may be said that facultative heterochromatinization is the result of regulatory processes, which cause repression of blocks of genes *en masse*; when the activity of these genes is needed again, the repression is withdrawn and the gene product is realized. Facultative heterochromatin illustrates the concept of co-ordinated control of chromosomal activity[30,33,129-131]. However, some points about the mechanism of heterochromatinization still remain enigmatic; for example, how does the cell decide or select between the two alternative chromosomes (homologous chromosomes) for condensation and inactivation? Once inactivation has been effected, what maintains it in subsequent cell generations? Answers to these and other questions would be required for a clear understanding of the mechanisms for the co-ordinated control of chromosomal functions.

The facultative heterochromatin is a means for differentiation. This regulatory nature becomes apparent on consideration of the structural and functional differentiation of sex-chromosomes in somatic and germ-line cells (Table 1). Sex-chromosomes are unique in that they carry genes for sex determination as well as other structural genes, apparently unrelated to sex determination or sex differentiation. Muller[132] suggested that X-chromosomes in *Drosophila* also carry compensator genes for dosage compensation genes for those X-linked genes which require equalized expression in the homozygous (XX) female and hemizygous (XY) male. Obviously, dosage compensation would not be required for sex determining genes, since it is the difference in their dosage that differentiates male and female sexes[132,133]. Thus, it is apparent that

TABLE 1 — HETEROCHROMATINIZATION AS A MEANS OF REGULATING THE ACTIVITY OF SEX CHROMOSOME IN SOMATIC AND GERM CELLS

Cell types	Mammals	*Drosophila*
(A) Somatic cells		
(i) XX (female)	One of the two X's randomly heterochromatinized-dosage compensation	Both X's remain euchromatic and normally active
(ii) XY (male)		
X-chromosome	Euchromatic, normal level of activity	Euchromatic-hyperactive-dosage compensation
Y-chromosome	Heterochromatic	Heterochromatic
(B) Germ cells		
(i) XX (female)	Both X's euchromatic-control of oogenesis	Both X's euchromatic-control of oogenesis
(ii) XY (male)		
X-chromosome	Heterochromatic	Heterochromatic
Y-chromosome	Activity (?) (sex vesicle)	Highly active, forms 'lampbrush' loops and synthesis of specific mRNA's-control of spermiogenesis

sex-chromosomes are required to function differentially in the somatic and germ-line cells of the two sexes[131].

In somatic cells of female mammals, one of the two X's is randomly heterochromatinized to achieve dosage compensation[105,106], but in cocytes, there is neither any allocycly nor genetic inactivity of the X-chromosomes, i.e. both the X's remain functional and euchromatic[131,134,135], since special 'sex-factors' located on the X are required to function for the cogonial differentiation. In male somatic cells, on the other hand, the single X remains euchromatic, while the Y remains heterochromatic. In testes, the X also becomes condensed, heteropycnotic and shows late replication and absence of transcription[136,139], since for spermatogenesis, activity of the X is not required[131].

In *Drosophila* and other insects too, a comparable situation exists[140,141]. Thus, in *Drosophila* female somatic cells both the X's remain euchromatic and functional, there being no inactivation of one X, comparable to mammals[59,142,143], but in males, the single X becomes hyperactive to achieve dosage compensation[143-149]. In oogenesis, the two X's become highly active and diffuse[147]. In spermatogenesis, the X-chromosome becomes heterochromatic and condensed, while the Y-chromosome which is completely heterochromatic in somatic cells, assumes a very active role in the spermatocytes, forming 'lampbrush' loops; the different regions of the Y-chromosome in *Drosophila* synthesize specific mRNAs for the process of spermiogenesis[150-153]. Table 1 summarizes the above observations, and these serve to emphasize that chromosomal morphology at a given stage in a cell type is the reflection of its functional state and not an inherent property. Earlier concept of 'once heterochromatin, always heterochromatin'[29] is obviously no more tangible. Such concepts have been misleading and have contributed to the misinterpretation of the nature and function of heterochro-

matin. What should be realized is the dynamicity of cellular and chromosomal activity and also the cellular economy. If a particular chromosome or part of chromosome were always inactive and functionless, why should the organism retain this unnecessary burden? But it seems natural that when a part of the genome is not required to function in a given cell or tissue, this may be heterochromatinized and thereby made unavailable to the transcribing systems of the cell. This is analogous to the repression of individual genes for the normal differentiation of an organism; only in the case of heterochromatin, groups of genes are simultaneously repressed by processes which are likely to be different from the individual gene regulatory systems (*see* also Section VI).

2. *Constitutive Heterochromatin*

(A) *General aspects* — Unlike facultative heterochromatin, constitutive heterochromatin is believed to be a 'permanently' inactive chromatin, devoid of functional genes and is believed to be unable to support transcription *in vivo*[154-156]. Recent studies, however, suggest positive functions for constitutive heterochromatin, mainly related to structural organization of chromosomes. Yunis and Yasmineh[5] have also discussed the nature and function of constitutive heterochromatin.

Typically, constitutive heterochromatin is localized near centromeres (pericentric heterochromatin), nucleolar organizer regions (nucleolar heterochromatin) and in some cases whole or parts of chromosomes are of this type. Sometimes, constitutive heterochromatin is also located as small to very small segments along the length of chromosomes (intercalory heterochromatin). Association of centromeres with constitutive heterochromatin now seems to be almost universal. All the examined species have shown the presence of constitutive heterochromatin and repeated DNA sequences[5,37,76,157-166]. The amount of pericentric heterochromatin varies with species from very small amounts to large blocks. Fig. 3 shows an example of large blocks of pericentric heterochromatin seen in *Rattus blan fordi*[167]. In interphase nuclei, the centromeric heterochromatin is often associated into large chromocentres (Fig. 2), and this points to some common property or homology of constitutive heterochromatin located on different chromosomes. This has been elegantly and adequately confirmed by *in situ* hybridization studies[5,157].

Nucleolar associated constitutive heterochromatin is also very common. Heitz[168,169] had earlier demonstrated a strong correlation between nucleoli and the satellite-bearing chromosomes. The nucleolar organizer regions express at metaphase as the secondary constrictions and such constrictions are always associated with constitutive heterochromatin. Recent studies have also demonstrated the presence of repetitious or satellite DNA with nucleolar DNA[5].

It is significant that rodents, known for their adaptive and genomic diversity[170,171], have a large amount of constitutive heterochromatin, and in many cases this is accumulated in specific chromosomes, especially the sex-chromosomes. Constitutive heterochromatin associated with sex-chromosomes is very interesting. In mammals, the X-chromosome is usually about 5% of the haploid autosomal complement, and when the X is larger than this,

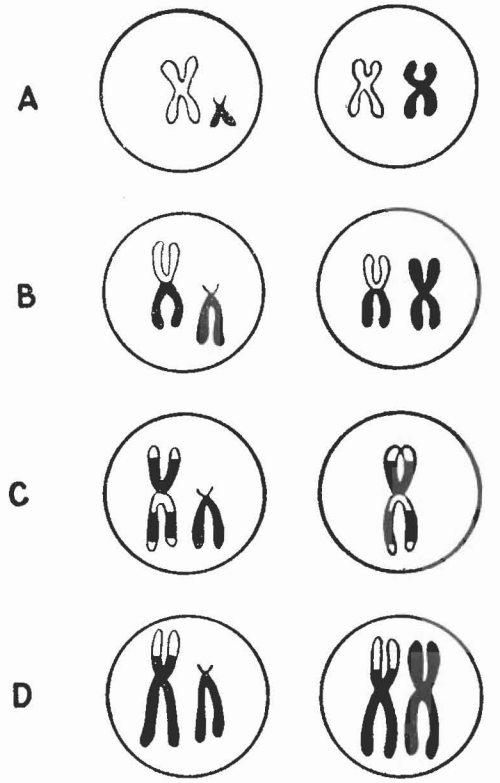

Fig. 6 — Sex-chromosomal heterochromatin in somatic cells of male and female somatic cells [Males on the left and females on the right side. Euchromatic areas on the X are outlined] [(A) The original 5%-type X; male with heterochromatic minute Y; female with one 'lyonized'-X; seen in most mammals; (B) duplicate-type X; male with one-half X and entire Y heterochromatic (constitutive); female with one and a half X heterochromatic (one half in each X is constitutive heterochromatin, in one X another half is facultative heterochromatin); seen in several rodents and some other mammals; (C) triplicate-type X; male with two-thirds X and entire Y heterochromatic (constitutive); female is normally XO, yet two-thirds of X is constitutive heterochromatin; seen in *Microtus oregoni*; (D) quadruplicate-type X; male with three-fourths of X and entire Y heterochromatic (constitutive); in female one entire X (three-fourths constitutive and one-fourth facultative) and three-fourths (constitutive) of other X heterochromatic; seen in *M. agrestes*. Note that as the X-chromosome increases in size, the Y-chromosome also increases correspondingly[29]]

all additional X-material (i.e. additional to the 'original' 5%) is constitutive heterochromatin[29,172] (Fig. 6). Depending on the relative size of the X-chromosome, 'duplicate', 'triplicate' or 'quadruplicate' type of X-chromosomes have been organized in several mammals. In all these cases, only the original 5% X-chromosome remains functional and euchromatic[29,30,173]. The largest X-chromosomes recorded in mammals are that of *Microtus agrestes* with the X about 30% of the haploid autosomal complement[174,176], and here, as expected, all but a small part of the X is heterochromatic. Another intriguing aspect of such large sex-chromosomes in mammals is the presence of a large Y-chromosome, associated with large X, and this too remains entirely heterochromatic[171,172].

Recently, Yunis and coworkers[5,178,179] examined the cytological variations in constitutive heterochromatin in different cell types and different developmental stages of *M. agrestes*. This animal is very suitable for such studies, since most of the consti-

tutive heterochromatin is located in the sex-chromosomes. It could be observed that in developing embryos as well as in diverse adult tissues and cell types, the constitutive heterochromatin of the giant sex-chromosomes is always condensed in interphase and shows the various characteristics of constitutive heterochromatin during cell division. Lee and Yunis[178,179] concluded that constitutive heterochromatin is a specific nuclear entity, and its elemental unit in interphase is a heterochromatic fibre, which can be packed or folded in any one of the set patterns, depending upon the cell type.

(B) *Constitutive heterochromatin and repetitive DNA* — The nuclear DNA of eukaryotes includes several classes of repetitive sequences of nucleotides[180], and some of these repetitive sequences, because of their unique base composition, form a 'heavy' or 'light' satellite band during isopycnic CsCl centrifugation, and these are termed 'satellite-DNA' compared to the main-band DNA[5,180,181].

Mouse satellite DNA is the best known example. Intranuclear localization of this class of DNA has been known only during the last few years. Yasmineh and Yunis[182-184] demonstrated the enrichment of satellite DNA in isolated mouse constitutive heterochromatin. Further studies using the technique of *in situ* hybridization have shown that the mouse satellite-DNA is located in pericentric heterochromatin of all the chromosomes[76,157].

In *M. agrestes*, the giant sex-chromosomes also have repetitive DNA in their constitutive heterochromatin, though the repetitive sequences do not form a major satellite in this species[185,186]. In other mammals, birds, amphibians, several Diptera like *Drosophila*, *Rhynchosciara*, etc., a close correlation has been obtained between the distribution of constitutive heterochromatin and repetitive or satellite DNA[5,45,76,157-166,181-197].

Recently, a simple method of Giemsa-staining of metaphase chromosomes after *in situ* heat or alkali denaturation, followed by renaturation at neutral *p*H, under controlled conditions, has been developed[37,38]. This method has been extensively used and many variants of the original technique have been developed[198]. Many species have been investigated, and in every case, the intensely Giemsa-stained regions correspond to the localization of repetitious or satellite DNA and constitutive heterochromatin. A typical case of such staining is shown in Fig. 7 for *Rattus blan fordi* chromosomes. The centromeric regions of all chromosomes and the proximal half of the X and entire Y stain intensely with Giemsa after denaturation and renaturation. Since such staining has been shown to be specific for repeated DNA sequences[37,38,45,199], it may be concluded that in *R. blan fordi* also, as in other mammals, the large X and the Y are rich in repetitious DNA. In snakes, the W-chromosome is known to be heterochromatic and to form a 'W-chromatin' in interphase[200], and this chromosome varies in size in different species. Although *in situ* hybridization studies have not been carried out, Giemsa-staining indicates that the W-chromosome is also rich in repetitious DNA (Singh and Ray-Chaudhuri, personal communication).

It may be emphasized that the repetitious DNA associated with constitutive heterochromatin is not a uniform entity. It is now abundantly clear that the nature and sequences of repetitious or satellite

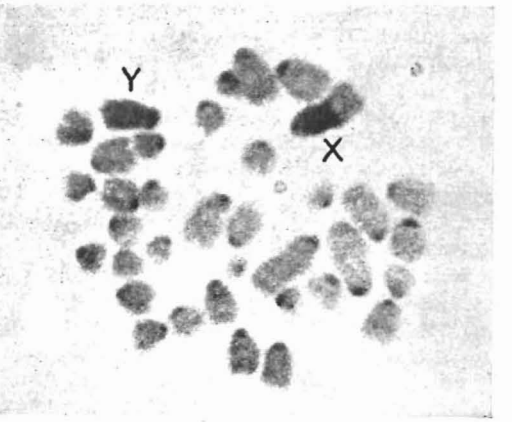

Fig. 7 - Constitutive heterochromatin in *R. blan fordi* chromosomes stained with Giemsa after denaturation and renaturation

DNA vary greatly even in the same or closely related species[5,166,185,191,192]. From a study on the DNA of mammalian and avian heterochromatin, Comings and Mattocia[192] suggested that several different categories of DNA may be associated with diverse heterochromatin fractions. The kinds of DNA listed are: (a) repetitious satellite DNA: (i) A-T rich, (ii) G-C rich, and (iii) with the same density as main band DNA; (b) repetitious main band DNA, and (c) non-repetitious DNA: (i) G-C rich heavy shoulder DNA and (ii) A-T rich main band DNA. It has been suggested that types (a) and (b) may be associated with centromeric heterochromatin, while type (c) DNA may be found in non-centromeric intercalary heterochromatin[45,165,166,191].

A further development of the Giemsa-staining for centromeric heterochromatin is the elucidation of metaphase chromosome bands or the banding pattern. A wide variety or techniques have been developed, but most of them employ denaturation and renaturation prior to Giemsa-staining[198,199,202]. Accordingly, it has been suggested that these darkly-stained bands represent the intercalary localization of repetitious DNA and heterochromatin. However, in another variant of this technique, trypsin is used instead of denaturation and renaturation. Thus, following a controlled digestion with trypsin, bands can be seen after Giemsa-staining[203-206], and these observations have been interpreted to indicate that the bands may not be produced because of repetitive DNA, but may be rather due to some local protein differences[205,206]. Another technique used to localize constitutive heterochromatin and bands involves the use of fluorescent dyes like acridine orange and quinacrine derivatives like quinacrine mustard (QM). Since the original discovery[39,40], this has been a very useful tool for localization and identification of heterochromatin. Recently, several attempts have been made to analyse the biochemical basis for intense fluorescence of certain chromosomal areas[166,207,208]. These studies indicate that the QM fluorescence is produced by A-T rich sequences[207,208]. Some workers, however, maintain that the base composition of DNA may be of minor or secondary importance in QM fluorescence, and once again, chromosomal proteins have been implicated in giving the specific fluorescent patterns[45,166,205,209].

An overall consideration of the various observations on chromosome banding, its relation to heterochromatin and repetitive DNA would greatly help in our understanding of chromosome organization. In every likelihood, the bands observed on metaphase chromosomes indicate local enrichment of repetitive sequences and constitutive heterochromatin. It is possible that the chromosomal proteins too have a linear differentiation along the length of chromosomes, and treatment with trypsin and other proteolytic agents can thus permit visualization of chromosomal bands due to differential and specific response of different chromosomal protein moieties to the treatments[206]. Further studies are needed along these lines.

(C) *Constitutive heterochromatin in Drosophila* — Constitutive heterochromatin in *Drosophila* provides a different aspect. As pointed out earlier, in *D. melanogaster*, proximal one-third of the X and entire Y are heterochromatic; besides, autosomal centromeric regions also have heterochromatic regions[210]. In polytene chromosomes, all heterochromatic regions fuse together to form a common chromocentre. Heitz[104] characterized the heterochromatin in *D. melanogaster* as α- and β-heterochromatin, and he suggested that heterochromatic regions fail to multiply during polytenic growth, resulting in an under-replication of these regions in the polytene nuclei. Measurements of the DNA content in polytene and non-polytene nuclei[58,211-213] have confirmed that the polytene nuclei do not have DNA in exact multiples of the non-polytene diploid nuclei. Studies with molecular *in situ* hybridization and specific Giemsa-staining have shown that both α- and β-heterochromatic regions of Heitz (both these can be grouped as constitutive heterochromatin of Brown[27]) contain repetitive DNA[158,159,161,162,195,210]. It has been shown that the DNA available for complementary binding with isolated repetitive DNA in the chromocentre of polytene nuclei remains nearly the same as in non-polytene nuclei[159,195], and it was concluded that while α-heterochromatin does replicate to some extent, β-heterochromatin fails to replicate during polytenization[159] (Fig. 8). Although α-heterochromatin does not replicate as a whole, the nucleolar organizer or the r-DNA located within this region[3,49] does replicate in polytene nuclei. This indicates that within the heterochromatic regions, some replicas may have their independent regulation[159]. A similar situation presumably exists in other *Drosophila* species with respect to heterochromatin organization.

The under-replication of heterochromatin in polytene nuclei is another example of differential replication of euchromatin and heterochromatin. However, this may be an entirely different kind of regulatory system than the usual late-replication of heterochromatin. Nevertheless, it is to be noted that in mitotic nuclei, the constitutive heterochromatin of X and Y in *Drosophila* are also late-replicating[215]. The Y-chromosome heterochromatin in *Drosophila* is interesting in other respects as well. It is widely believed that heterochromatin in general and constitutive heterochromatin in particular is genetically inert and does not transcribe *in vivo*. However, *Drosophila* Y-chromosomal heterochromatin is one instance where specific functions at a specific stage of development could be attributed, namely transcription of specific mRNA's to control spermiogenesis[150-153]. Different regions of the Y-chromosome have been shown to have specific functions in regulating spermiogenesis[150-153]. While

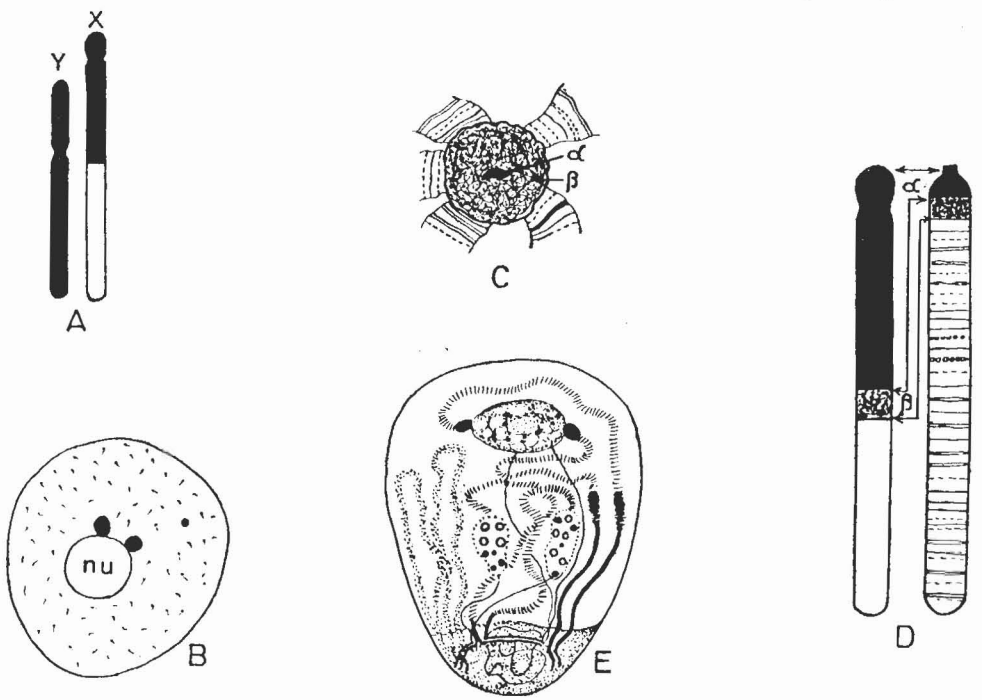

Fig. 8 — X- and Y-chromosomal heterochromatin in *Drosophila* [(A) Mitotic chromosomes of *D. melanogaster*; entire Y and proximal one-third of X heterochromatic (shaded); (B) X- and Y-chromocentres in mitotic interphase nuclei; two chromocentres associated with nucleolus (NU); (C) chromocentre in salivary gland nuclei; alpha and beta heterochromatin may be visualized; Y-chromosome remains buried in the chromocentre; (D) schematic comparison of the relative content of alpha and beta heterochromatin in mitotic (left) and polytene (right) X chromosome. Alpha and beta heterochromatin show different degree of under-replication in polytene nuclei[159]; (E) Y-chromosome loops in *D. hydei* spermatocytes, active in RNA synthesis[151]]

the Y-chromosome is so essential for sperm differentiation, it has no function and activity in somatic cells and can be completely eliminated from these cells without any deleterious effects: the XO males without a Y-chromosome are perfectly viable, though sterile (*see* Section II). Thus, *Drosophila* Y-heterochromatin expresses itself differently in different cell types. In normal somatic cells, it remains constitutively heterochromatic, in polytene nuclei it fails to replicate, while in spermatocytes Y-chromosome undergoes unfolding and an intense period of RNA synthesis (Table 1 and Fig. 8). All these aspects suggest that the Y-heterochromatin of *Drosophila* (and possibly of other organisms as well, for example *see* Zuk[216], for the behaviour and properties of the Y-chromosome in *Rumex*) is a distinct entity endowed with specific functions. It may be significant that Y-chromosome of *D. melanogaster* is rich in poly dAT[217].

V. Biochemistry of Heterochromatin

During the last decade, techniques have been developed for isolating different chromatin fractions from nuclei in more or less native state, and this has permitted their biochemical characterization. Recently, Frenster[52] has discussed in detail the biochemical aspects of heterochromatin and euchromatin.

(1) *DNA in heterochromatin* — It has been seen above that the DNA in constitutive heterochromatin is usually unique in showing repeated base sequences and such repeated sequences are often expressed as satellite DNA, having a base composition different from that of the bulk of DNA in euchromatin.

However, leaving aside the case of repeated DNA and constitutive heterochromatin, the overall base sequences in other kinds of condensed chromatin are not expected to be different from that in euchromatin. Thus, facultative heterochromatin, obviously, is not different in base composition from its homologue, which remains euchromatic. In fact, in earlier studies, when the techniques for characterizing and localizing repeated DNA were not developed, it was generally believed that the DNA from euchromatin and heterochromatin do not grossly differ in their base composition, thermal denaturation, or in their reactivity to anti-DNA-antibodies[52,118,219]. Thus, facultative heterochromatinization, *per se*, does not lead to changes in base sequences or genetic constitution.

(2) *RNA in heterochromatin* — Heterochromatin differs from euchromatin in its RNA content and capability to transcribe RNA. Dolbeare and Koenig[220] found that the diffuse and condensed chromatin isolated from rat liver nuclei contains about 2·5 times more RNA than the rat liver nuclei, though the condensed chromatin contains about 75% of the nuclear DNA. Comparable results have been obtained by Frenster[52]. *In vitro* analysis of the transcription in isolated condensed chromatin has shown that it does not support active RNA synthesis and incorporates little or no RNA precursors[51,220-222]. In the mealy bugs, removal of histones results in almost equal transcription rates in euchromatin and heterochromatin[128,223].

These *in vitro* observations confirm the observed inactivity of heterochromatin *in vivo*. Many studies have demonstrated that in living cells also, both facultative and constitutive heterochromatin fail to incorporate ^{3}H-uridine[53,54,154-156,223].

In several studies, ^{3}H-actinomycin D (AMD) has been used to analyse the transcriptive ability of chromatin, since it is believed that AMD binding is correlated with the degree of RNA synthetic activity[223-225]. Facultative heterochromatin in coccids was found to bind much less AMD than the euchromatic chromosomes[223]. However, not all condensed chromatins respond similarly to the binding of AMD. Constitutive heterochromatin, in particular, binds AMD almost as much as or even more than euchromatin[156].

Thus it seems that while all categories of heterochromatin are inactive in transcription, the mechanisms by which this is brought about may be different for different categories. This does not, however, imply that the heterochromatic regions always remain inactive; they are inactive only during their heterochromatic state.

(3) *Proteins in heterochromatin* — Analysis of chromosomal proteins has been done by several groups of workers in different systems[51,52,128,218,220,222,223,227-229]. Studies on coccids have indicated a differential role for histones in the euchromatic and heterochromatic chromosome sets[51,223,226]. It has been shown that removal of histones results in decondensation and restoration of transcriptive activity of the heterochromatin chromosomes[223,226]. It has also been suggested that there is a greater post-synthetic acetylation of histones in euchromatin than in heterochromatin in mealy bugs[226].

In most systems, however, no qualitative and/or quantitative differences could be established between the histone content of euchromatin and heterochromatin[50,220,222,227,229]. But a quantitative difference has been observed for the content of non-histone residual proteins, phosphoproteins and phospholipids, as euchromatin carries 2-5 times more of these than heterochromatin[54,220,229]. This higher concentration of acid proteins is correlated with the higher transcriptive activity of euchromatin and the relative inactivity of heterochromatin.

It seems, then, that eu- and heterochromatin differ not only in their acid protein content but also in the organization of DNA and histones. Of course, these two aspects could be interdependent. In heterochromatin, the histones could probably be associated with DNA in a way that does not permit template activity, but addition of acid proteins may modify this interaction.

VI. Significance of Heterochromatin

The recent exciting discoveries in molecular biology of heterochromatin necessitate a complete reassessment of the nature and significance of heterochromatin. Some basic questions still remain unanswered. Is heterochromatin really inert or functionless? How do we characterize heterochromatin? Why do some species have very little and others a very high amount of heterochromatin? Is it dispensable? Satisfactory answers to these and other questions may not be available with our present state of knowledge on chromosome organization and function. Nevertheless, some apects are considered here with a view to stimulating further thinking and experimentation.

The characterization of heterochromatin is considered first. Condensed state and apparent lack of genetic activity are the two basic attributes of heterochromatin. However, this needs further

qualification. At metaphase, all the chromosomes are maximally condensed and are almost inactive in RNA synthesis. This certainly does not mean that all metaphase chromosomes are heterochromatic! Heterochromatin is a relative term. When some part of chromatin behaves differently from the rest, the concept of heterochromatin comes in. The condition in polytene chromosomes illustrates a different aspect of heterochromatin characterization. The nuclei are in permanent interphase[230] and are linearly differentiated into bands and interbands. The bands are the condensed regions and are relatively inactive in RNA synthesis[230,231]. At times, some bands decondense and begin active transcription. It may be asked then: should these bands also be considered as heterochromatic? These condensed and inactive bands may be analogized to the heterochromatic chromosomes in coccids, for example. In condensed state they are inactive, but when decondensed, they become active in transcription. However, some very important differences between the two systems exist.

In the case of bands, both the homologues are condensed or decondensed, while in coccids, only one haploid set is inactivated. Besides, the chromosomes of coccids are condensed and inactivated *en masse*, i.e. a unified signal probably elicits heterochromatinization in all the target chromosomes (supragenic regulatory system). In the case of bands of the polytene nuclei, the activity and condensation of different loci are controlled not by a single stimulus, but by individual respective regulatory genes[233]. In non-polytene cells too, a similar mechanism exists. In any given cell, only a small fraction of genes are active in transcription, the rest being inactive and condensed[52,221,234]. Such inactivation of specific genes in a given cell type is a sequel to the process of differentiation and development. Do we consider these inactive genes, which form the bulk of the chromatin of a cell, as heterochromatin? They share with heterochromatin the properties of transcriptive inactivity, condensed state, and in some instances, possibly late replication also. However, it is important to note that the mechanisms by which such inactivation is brought about in heterochromatin and repressed genes are different.

The term heterochromatin may, with advantage, be restricted to those instances where a supragenic level of control is involved in bringing about condensation, the apparent lack of activity and other correlated manifestations. With this limitation, the genes that are individually inactivated in development would not be considered heterochromatic. This criterion may apply equally well to the large blocks of heterochromatin at centromeres and other locations, the intercalary heterochromatin and the facultative heterochromatin.

It is implicit that heterochromatinization is also the product of the regulatory processes operating in the cell and, therefore, all kinds of heterochromatin have the potentiality to revert to the so-called euchromatic state or to be genetically active. Facultative heterochromatin, of course, is well known (*see* Section IV.1) to be either inactive or active and euchromatic in different cell types of an individual. With regard to the constitutive heterochromatin, the opinions vary. It is usually believed that constitutive heterochromatin is inherently inactive with respect to the known mechanisms of gene function, i.e. constitutive heterochromatin does not carry the so-called 'Mendelian genes' and cannot transcribe mRNA[156]. Consistent with this notion is the association of constitutive heterochromatin with simple repetitive DNA, which is unlikely to code for any protein[235]. However, several aspects need to be reconsidered before concluding that constitutive heterochromatin is inert in gene activity.

Genetic activity of DNA is usually studied in terms of transcription of specific RNA molecules, viz. mRNA, rRNA, tRNA. Is it necessary that DNA exert its influence only through these RNA molecules? Probably not. Products of regulatory genes may not be translated into proteins, but the regulatory action is still achieved. Besides, there are indications that much of the nuclear RNA never reaches cytoplasm[129,236-242]. These observations indicate that some DNA in the genome may function in ways other than the classically established pathways of transcription and translation. Constitutive heterochromatin DNA may be an example in this category.

The transcriptive inactivity or inability of constitutive heterochromatin or repetitious DNA is also not universally accepted[196]. Flamm *et al.*[243] could not find any RNA complementary to the isolated satellite DNA of mouse. Similarly, Sieger *et al.*[155,156] did not find evidence for transcription of constitutive heterochromatin *in vivo* in *Microtus agrestes*. On the other hand, some evidence for the transcription of the satellite sequences *in vitro* has been presented[241,245]. In this context, the observations on the centromeric heterochromatin in *D. melanogaster* and the satellite DNA of mouse are very interesting[196,245]. Evidences have been presented to indicate that the repeated sequences in *Drosophila* constitutive heterochromatin and the mouse satellite DNA are not simple tandem repeats[196]. Interspersed between the simple repeated sequences are more complex sequences, which have been provisionally called the 'spacer' DNA. These 'spacer' sequences could bear important genetic information, and the satellite DNA may be the true spacers for these sequences. Walker[181] suggested that the satellite sequences might have originated by duplications of ribosomal spacer sequences. Thus, it may be that while the satellite or repetitive sequences in constitutive heterochromatin of centromeres are nonsense, as far as coding for proteins is concerned, the newly discovered interspersed sequences[196] may have vital information. This model for centromeric heterochromatin may be compared to the arrangement of ribosomal cistrons in the nucleolar-organizer region heterochromatin, where the consecutive ribosomal sequences are spaced by small sequences, which apparently do not transcribe. Transcription of these interspersed complex sequences in the centromeric heterochromatin of *Drosophila* and mouse has been indicated[196]. The parallel between the arrangement of functional sequences in the nucleolar-organizer region and the centromere is significant, since both the regions are classically considered as constitutive heterochromatin. It may be that all constitutive heterochromatic regions have their functional cistrons buried in repetitive or satellite sequences. The model of constitutive heterochromatin with repetitive

sequences serving as spacers for some other sequences provides a basis for the proposed specialized chromosomal 'housekeeping' functions[5,180,181,196] of constitutive heterochromatin.

Britten and Davidson[238] have presented a very interesting model of gene regulation in higher organisms. It is postulated that every cistron has some repetitive sequence at the initiation point and that the variability and other aspects of these provide a plasticity for gene regulation and modulation (*see* also Georgiev for a similar concept[239]). Recently, evidence has been presented that *Drosophila* genome contains, on an average, a short, about 150 base pairs long, middle-repetitive sequence per approximately 750 base pairs of the single copy DNA[242]; this observation is strikingly similar to the postulation of Britten and Davidson[238]. If this distribution of repetitive sequences holds true in general, which seems likely, we have a genome organization, in which the repetitive sequences play very important roles in the regulation of 'euchromatic' genes. We have also seen that in constitutive heterochromatic regions too, there are 'spacers' of repetitive nature which may separate more important (probably unique) sequences. Eicher[33] has suggested a similar model with 'starters' and 'terminators' for each gene and a unified control of all these for inactivation in facultative heterochromatin. Thus, it may be that the whole genome of the eukaryotes is organized with 'spacers' (which probably are of repetitive nature) in between the important functional genes. These spacers may serve to control the initiation, termination and modulation of transcription of the associated functional cistrons. The nature of spacers may vary in hetero- and euchromatin. In heterochromatic regions, especially the constitutive heterochromatin, the spacers may be of a different degree of repeatedness, so that the entire region is switched off by a single stimulus.

In one instance of the constitutive heterochromatin, transcriptive activity for specific functions is known. This is the Y-chromosome of *Drosophila*, which has already been discussed in Section IV.2.C. This again shows that the constitutive heterochromatin is not inherently inert or incapable of transcription. The information in the constitutive heterochromatin may be for some very specialized functions and events, as for example, the spermiogenesis in the case of *Drosophila* Y-heterochromatin. The specialized nature of information requires that the constitutive heterochromatin be active for only very brief periods and possibly only in some cell types, depending on the nature of information carried in its different segments. It may be conceptual and technical limitations only, which do not permit us to understand and 'see' the activity patterns of constitutive heterochromatin, when in reality they are there. Like the facultative heterochromatin, the constitutive heterochromatin may also be considered as repressed chromatin, the action of which can be realized upon withdrawal of the repressive factors.

Accepting that heterochromatin does have functional roles in the cell, some possibilities may be explored. Different workers have suggested various 'functions' for heterochromatin. However, only recently the bases for these supposed functions are being understood. The facultative and constitutive heterochromatins, though similar in many of their responses, should be considered separately with regard to their roles in cell function, since it is clear now that the two are fundamentally different in their organization and functions.

Facultative heterochromatin probably does not have positive roles in chromosome activity. It is the absence of genetic activity in the facultatively heterochromatinized chromosome regions that is important in cell differentiation. For example, the 'inactivity' of one X in somatic cells of female mammals serves for dosage compensation. Similarly, other examples of facultative heterochromatin may be playing their role in differentiation and development through inactivity. However, it is questionable whether the facultative heterochromatin is completely inactive. There are suggestions that the 'Lyonized-X' in mammals may be only partially inactive[33,34,127]. Besides, it is also possible that the heterochromatic state itself may have its own regulatory or other kind of action.

Constitutive heterochromatin has been implicated in a variety of functions. Majority of these can be grouped together as chromosomal 'housekeeping' function[5,181]. The almost universal association of constitutive heterochromatin with centromere suggests, *a priori*, that it plays important roles in (i) chromosomal movements in mitosis and meiosis; (ii) pairing of homologous chromosomes; and (iii) maintenance of chromosome integrity, etc. Chromosomal movements during karyokinesis are very precise and coordinated. Obviously, these are under a strict control. Centromere is indispensable for these events, since acentric fragments often fail to manoeuvre themselves for orderly segregation. Constitutive heterochromatin, with its repetitive and other kinds of sequences (e.g. the 'spacers' of Kram *et al.*[196], discussed above) may provide the attachment sites for the spindle fibres. Ultrastructural studies of centromeric regions have shown a very specialized structural organization of the kinetochore[246-248]. The relationship between these structural features, constitutive heterochromatin and repeated sequences, remains to be understood. Brinkley and Stubblefield[246] suggested that the kinetochore DNA may transcribe special RNA's at specific moments in cell cycle, which may code for the spindle proteins or other structural features involved in chromosome movements during cell division. It is interesting to speculate that the so-called 'spacers' of Kram *et al.*[196] may have the necessary information for some or all of these events. It is worth noting that polytene nuclei, where the chromosomes multiply but do not segregate, have almost dispensed with centromeric heterochromatin (*see* Section IV.2.C). Is it possible that since these cells do not require the information for spindle organization and associated events, the DNA controlling these functions is not replicated for the sake of cell economy? It will be interesting to examine the status of centromeric DNA in those polytene nuclei which can also transform into polyploid nuclei; such nuclei would be expected to retain the centromeric DNA in the same proportion as the rest of it.

In addition to the involvement in centromeric activities, constitutive heterochromatin also serves to prevent recombination in some very important and vital sequences, like ribosomal cistrons. No

chiasmata are formed in constitutive heterochromatin[77,249,250], and this prevents recombination of genes located in constitutive heterochromatin. Alterations in the base sequences in some cistrons, like ribosomal- and transfer-RNA's, may be deleterious, and these sequences have remained essentially the same throughout the live forms[251]. Heterochromatin might be essential in eukaryotes to prevent recombination and spread of mutations in these sequences[5,102].

Summary

Different aspects of the nature and constitution of heterochromatin are discussed. Facultative and constitutive heterochromatins are explained at length and their properties — genetic, biochemical and structural — are described. The characteristics of these heterochromatins have been correlated with their functional behaviour and their roles during differentiation and cell replication at chromosomal and molecular levels have been discussed in the light of recent advances. Some aspects of the position effect of heterochromatin have also been considered. The susceptibility of heterochromatin to certain treatments and chemicals, and its significance with respect to the structure and function of heterochromatin have also been discussed.

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