HETEROCHROMATIN IN PERSPECTIVE

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ABSTRACT

A discussion on the understanding and the status of heterochromatin as it is viewed today, particularly in relation to constitutive heterochromatin variability and the possible significance in chromosome mechanics, function, organisation and behaviour is presented. Some unresolved aspects about the heterochromatin are visualised and the future prospects are enumerated. The applications of banding techniques as a probe to investigate this important phenomenon and its control mechanisms are suggested for a better understanding citing suitable specific examples for further research.

INTRODUCTION

THE term heterochromatin has been used by various authors to decribe very diverse phenomena. Heitz used this term to denote chromosome regions that were condensed in interphase and prophase and do not decondense or unravel in telophase like the rest of the chromosome[1]. The heterochromatin can be divided into two basic types[2]. In *facultative* heterochromatinization, the two homologous chromosomes differ, one becomes heterochromatic during development and the other remains euchromatic (*i.e.* the mealy bug type). In *constitutive* heterochromatinization, both the homologous chromosomes, one maternal, the other paternal, respond in the same way during development.

The *constitutive heterochromatin* is in most cases characterised at least by (a) its condensed (compacted) state during interphase forming what is called as chromocentres, (b) late replication of DNA, (c) faster replication of DNA than in euchromatin and transcription inactivity[2,3]. Additionally, this type of heterochromatin has frequently been found to be rich in satellite or repetitive DNA and to be preferentially involved in spontaneous or induced chromosomal structural changes[4].

Recent introduction of chromosome banding techniques have unravelled the heterochromatic segments including the repeated DNA sequences on chromosomes at all the stages of cell cycle and have thrown considerable light on chromosome structure, organization, behaviour and evolution[5]. Information is presented on some more aspects of constitutive heterochromatin variability and behaviour. A need for further research is visualized for a clear understanding on the nature of heterochromatin.

CONSTITUTIVE HETEROCHROMATIN VARIABILITY

A. *Polymorphism at population level*: Several instances of variability in constitutive heterochromatin and/or their associated highly repetitive DNAs have been cited[4]. Polymorphism of C-banding patterns in chromosomes has been shown in a variety of animals and plants[6-8]. Existence of extensive C-band polymorphism in pairs of homologous chromosomes which otherwise pair and seggregate normally, have recently been stressed[9] and the phenomenon has mainly been attributed to variation in chromosomal DNA content.

Following the relationship between constitutive heterochromatin and repetitive DNA, such variants could hypothetically arise through selective amplification[10] of repetitive DNA and their subsequent distribution among chromosomes by means of unequal crossing over and translocation.

B. *Variability at species level*: Introduction of banding techniques have facilitated the study of finer details of chromosomes and has provided a valuable tool for tracing species relationships[11,13]. Variation in patterns of constitutive heterochromatin are reported and made use of in the evolution of karyotype and species due to structural alteration or due to chromatin diminution, particularly of heterochromatic fraction.

The analysis of complements of highly repeated DNA sequences in *Drosophila* and cereals show that the length of tandem arrays of a repeat, rather than its nucleotide sequence, is the evolutionary variable in heterochromatin structure[14,15]. Thus any model for evolution of satellite DNA must encompass a mechanism for amplifying a DNA segment into a longer (or reducing it to a shorter) tandem array of these segments[14].

C. *Variability during ontogeny:* Organ to organ variation in DNA content in the active nuclei of various plant organs has been shown[16]. It has been suggested that the amplification and diversification of

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regulatory DNA sequences are molecular bases of speciation and organogenesis. Further, it has been found that organisms show much variation in nuclear DNA during development and differentiation[16-18].

Ontogenetic DNA variation parallels phylogenetic DNA variation in most cases, if not always, heterochromatin or at molecular level, repetitive and satellite DNAs are involved. Heterochromatin underreplication has been shown by cytophotometry in insects[19] and angiosperms[20,21].

Heterochromatin amplification in somatic cells is known from salivary glands of sciarid flies, and has been recently found in protocorms of the orchid *Cymibidium*[17,22]. Extra replication of satellite DNAs has been reported to occur in tissue cultures of *Crepis*[23] and *Nicotiana*[24] and the giant suspensor cells of *Phaseolus*[25].

D. *Variability in B chromosomes*: The redundancy of heterochromatin, resulting in compaction and shortening of chromosome size, can be extended to the behaviour of accessory chromosomes which represent mostly a cytological embodiment of heterochromatin and show analogous behaviour[26]. The buffering action and adaptive advantage of B chromosomes (majority of B chromosomes are heterochromatic in nature), through their effect on recombination and other aspects of metabolism have been indicated. In *Allium stracheyii* of the Eastern Himalayas, the occurrence of upto 8B chromosomes in diploids and their complete absence in the polyploid individuals of the same species was recorded[27]. These diploid individuals, when grown under tropical conditions, were gradually converted into polyploids with the complete elimination of B chromosomes. Induction of polyploidy and subsequent elimination of Bs could be avoided by growing plants in artificial environment with lower temperature simulating the Himalayan conditions[27]. SubbaRao and Pantulu[28] using special addition lines in pearl millet having derived B chromosomes in normal plants have suggested that extra heterochromatin included due to addition of Bs has a repressing effect on mean chiasma frequency as compared to the addition lines with euchromatic Bs which have enhancing effect on chiasma frequency.

Possible Role of Constitutive Heterochromatin

A. *Heterochromatin/satellite DNA/chromosome pairing*: The hypothesis that satellite DNA is involved in some aspects of chromosome mechanics such as homologue recognition and pairing at meiosis is a very common one and is widely accepted[29-31]. A large amount of circumstantial evidence exists in the literature on the other hand concerning this interpretation, suggesting the pairing function of satellite DNA as extremely unlikely[32-35]. Yamamoto[9] clearly demonstrated for the first time in male *D. melanogaster* that the highly repeated satellite DNA sequences in heterochromatin are not important for autosomal chromosomal pairing. However, the findings that (a) deletion of X chromosome heterochromatin in *Drosophila* systematically modifies the inhibitory influence of the centromere on meiotic recombination[34], (b) heterochromatin which exists as segments or supernumeraries directly affects the recombination system[36] and (c) telomeric satellite DNA can also function in altering recombination[37] imply that one function of satellite DNA on which heavy data is now available is its crucial involvement in the meiotic recombination system. The authors[33,35] argue strongly for a role of heterochromatin in germ line rather than in somatic cells. Heterohromatin, therefore, may be dispensable for the survival of a cell or an individual, but it is essential for the survival of a chromosome in the germ line[38].

Merker[39] using isogenic lines with the presence and absence of the telomeric heterochromatic block of rye chromosomes in an advanced hexaploid triticale line observed that the plants with added heterochromatin affect triticale lines and disturb the meiotic behaviour. Further, Merker[40] suggested that the selection of triticale lines with reduced amount of heterochromatin may regularise the meiotic behaviour and thus add to cytological stability.

The relation of chiasma to heterochromatin and the occurrence of chiasma terminalisation have been controversial problems in cytogenetics; however, it is only since chromosome banding methods are available that these problems can be investigated more accurately. There is good evidence that crossovers seldom, if at all, occur within, heterochromatin[41,42]. However, preferential occurrence of chiasmata near C-bands was reported in rye[42]. Loidl[43] has investigated both the occurrence of chiasma terminalisation and the relation of chiasma to C-bands in male meiosis of *Allium flavum* from early diplotene to metaphase I and suggested conclusively that chiasmata occur almost exclusively very close to terminal and intercalary C-bands. The majority of exchanges occur in euchromatic segments inserted into heterochromatin blocks. Using heterozygosities of partner chromosomes with regard to the presence or absence of intercalary C-bands it was suggested that here chiasmata do not terminalise and remain almost exclusively C-band associated.

B. *Relation between the SCE points and heterochromatin bands:* Differential staining of sister chromatids using FPG technique has allowed the identification of sister chromatid exchanges. Using two step staining for simultaneous production of Q or G bands and sister chromatid differentiation, it was found that sister chromatid exchanges occur more frequently in interband regions as compared to

heterochromatic segments[44,45]. Hoo and Parslow[46] described a method of sister chromatid staining in combination with DNA replication banding and suggested that SCEs occur about 3 times more frequently in early DNA replicating regions (i.e., euchromatic segments) as compared to late DNA replicating regions (i.e., heterochromatic segments). This preferential involvement of the euchromatin rather than the heterochromatin may be due to the more condensed and rigid nature of heterochromatin preventing the rotation of sister chromatid in case of breakages in this region.

C. *Chromosome breakage and heterochromatin*: In a variety of organisms using chemicals and physical agents, it has been found that heterochromatic segments are more susceptible to chromosome breakage[4,47].

It has been further observed that chemicals, *e. g.* mitomycin C in mammals [48,49], MH in *Vicia faba*[50] specifically break certain heterochromatic segments. This nonrandom distribution of breakage-rejoining points suggested that the hot spots for chromosome-breakage-rejoining are the sites of constitutive heterochromatin and some repetitive DNAs as noted through banding techniques. Observation like these led Hsu[51] to propose a "bodyguard hypothesis" for constitutive heterochromatin. According to him, constitutive heterochromatin is used by cell as a bodyguard to protect the vital euchromatin by forming a layer on the outer surface of the nucleus. Mutagens, clastogens or even viruses attacking the nucleus must first make contact with the constitutive heterochromatin which bears the assault, thus sparing the euchromatin from damage, unless the detrimental agents are overpowering.

D. *Role of heterochromatin in the control of cell cycle:* It has been suggested that DNA contents have increased during general evolution[52] but have decreased during specialisation[53,55]. Nagl[56] studied the relationship between nuclear DNA content, percentage of heterochromatin and cell cycle duration in the genera *Anacyclus, anthemis* and *Artemisia* and found evidence that the amount of heterochromatin per genome is related to the reduction of cell cycle times in annual plants with large DNA content. The results indicated that the nuclear DNA content can increase by the addition of heterochromatin without lengthening the cell cycle. The cycle may even become shorter. The percentage of heterochromatin within a given genome might, therefore, represent a correcting factor in the control of the duration of the cell cycle, in addition to the nuclear DNA content (the basic determinant). Thus the amount of heterochromatin is evidently an important factor in determining both nuclear DNA content and growth rate parameters. To test this hypothesis Bösen and Nagl[57] worked out cell cycle duration in mitotic and Caffeine induced binucleate endomitotic root tip cells in heterochromatin rich monocot *Allium carinatum* and confirmed the role of heterochromatin in shortening of cell cycle duration and suggested a possible advantage of the endomitotic cycle for rapid cell and tissue growth.

E. *Heterochromatin in chromosome movement:* One property of heterochromatin noted in many species is neocentric activity[41]. A neocentromere is cytologically defined as region of chromosome arm, other than the regular centromere, which can at a certain time display an apparent active role in polar movement on a mitotic or meiotic spindle. Neocentric activity has frequently been described in interspecies hybrids[58]. In general it is restricted to heterochromatic regions of chromosomes[41]. Lindsley and Novitaski[59] noted in *Drosophila melanogaster*, that heterochromatin adjacent to centromeric regions of chromosomes does help in adding to the kinetic activity of chromosomes to enhance the anaphase movements. In *Lathyrus cicera* we have noted that some of the cells in root tip tissue are tetraploid, where a slight variation in banding pattern is noted as compared to normal diploid cells in the same root. Particularly in some of the chromosomes of 4n nucleus the heterochromatin in centromeric regions was not discernible (our unpublished observations) suggesting thereby that heterochromatin may have some significance in chromosome movement.

F. *Application of heterochromatin recognition in understanding the spatial relationship of chromosomes:* Under normal conditions chromosomes cannot be identified during interphase or late anaphase-telophase. Recognition of heterochromatic segments can to some extent help in identification of particular chromosomes at such stages. Taking help of banding techniques information has been gathered on somatic association of chromosomes during interphase and its application in working out homologous and nonhomologous relationship, behaviour and arrangement of centromeres, telomeric fusions, orientation of interphase chromosomes and nature of interchromosomal connections. The information could be used to understand the basic arrangement of chromosomes and the spatial relationship of the genetic apparatus within itself and in relation to nuclear membrane[60,61].

G. *Chromosomal distribution of constitutive heterochromatin:* Chromosome banding techniques during the last few years have allowed the analysis of heterochromatin banding patterns on the chromosomes, suggesting that the distribution of chromosome bands is species specific. Banding patterns have been identified in procentric, pericentric, telomeric and intercalary regions. Even in the same genus the various types of banding patterns have been identified and their possible mode of

distribution in phylogen and evolution have been discussed *e.g.* *Lathyrus* spp. where a gradual diminution in intercalary, terminal bands has been recorded during the course of evolution[13]. Similarly the banding patterns have significantly helped in genome analysis of wheat and probable genome donors have been identified on the basis of specific distribution of heterochromatin in ancestral and present day forms of wheat[62,63].

H. *Hybrid induced changes:* When chromosomes containing blocks of heterochromatin from one species are introduced into the genetic background of another, these blocks can undergo an immense increase in size. For example, *Nicotiana otophora* possessing large heterochromatic segments on its chromosomes when crossed with *Nicotiana tabacum* having only scattered segments of heterochromatin in its genome, megachromosomes upto 15 times normal length are found in F_1. Such giant chromosomes are formed as a result of enlargement of the large heterochromatic blocks found in *N. otophora*[35].

Problems and Prospects

In the light of the foregoing information, the heterochromatin behaviour and organization of hetero chromatin poses so many questions which warrant conclusive answers and may throw considerable light in understanding the constitutive heterochromatin. A brief background and possible suggestion regarding the unresolved aspects are given below for reappraisal and investigation.

(a) Apparently wide natural or induced variability in certain blocks of constitutive heterochromatin may be tolerated by certain cells[14]. The limits of this variability and to extent these changes are associated with any modification or alteration in cell function are to be studied. It is likely that a genome has a certain amount of tolerance for this biological moiety, so that parts of it could be lost or gained without any detectable malfunctioning. Also more variation could be induced and be tolerated by cells cultivated *in vitro*. Therefore, studies on the variability in constitutive heterochromatin, coupled with its constituent repetitive DNAs in different cell populations of a species may determine the tolerance of the genome for such variants and limits of this tolerance for more variability. These regions of constitutive heterochromatin may be interspersed within genes for essential sequences.

(b) Variability in chromosome constitution and C-band variability is noted in cells cultivated *in vitro* at various lengths of time. The study on C-band variability may determine the role of heterochromatin in the control of cell growth, chromosomal stability and adaptability in culture which is specifically needed as a minimal requirement for cell survival. At the same time, the variation in heterochromatin of a cell line *in vitro* may affect and determine its cell cycle duration and in turn the rate of cell growth which could be used for basic and applied purposes.

(c) Recent researches have revealed that chromosome constituents change their pattern during different phases of development, maintaining of course, a basic genetic make up responsible for hereditary stability[17,26,64]. From the information available it would be desirable to know the pattern of DNA variation, whether it is due to under or extra-replication of heterochromatin. Chromosomes from such tissues where polytenic chromosomes are found *e.g.* suspensor cells in plants, salivary glands in insects or at the places where cells behave endomitotically at the time of differentiation of organs, the distribution and amount of heterochromatin may provide some information on the expression of character and organogenesis.

(d) The suggestion that constitutive heterochromatin may help to recognise homologues for pairing could be suitably substantiated undertaking studies on heterochromatin rich material and its impact on somatic pairing, observing the pairing behaviour and frequency in such materials which significantly differ in their heterochromatin at population level in the same species as noted in insect species. Sachan and Tanaka[65] observed variation in heterochromatin content and C-banding pattern in some primitive and present day races of *Zea mays*. Similarly, Vosa[66] noticed significant polymorphism for heterochromatin patterns and amount on all the chromosomes in a population of *Scilla sibirica*. Study of pairing behaviour of such material taken in conjunction with C-banding meiotic analysis may throw significant light on pairing phenomenon and constitutive heterochromatin. Interracial hybridization may also help generate high heterozygosity for banding patterns, which could be utilised in various ways including its application in homologue recognition, chiasma terminalisation in relation to heterochromatin.

(e) Evidence that C-band regions are not involved in chiasma formation, but at the same time chiasmata are proximal to C-band regions, need to be investigated on a wide range of plants and animals, using variety of banding patterns including terminal, intercalary and centromeric C-bands. More information should be gathered on the role of heterochromatin in chiasma terminalisation using material heterozygous for certain heterochromatic segments and its implication on chiasma formation, and points of exchange in such segments, before generalizing this assumption on the basis of test suggested by Loidl[43]. The relation of recombination with amount, distribution and chemical composition (AT or GC richness of DNA, which could beclassified

using Q-banding technique) of C-bands will have to be investigated on a larger scale with particularly suitable chromosome variants.

(f) Merker[40] observed in Triticale that the meiotic pairing is significantly increased which in turn improves the seed fertility, if the selection of the parents before the production of hybrid material of Triticale (crossing *Secale cereale* and *Triticum aestivum)* is made for reduced amount of C-band heterochromatin. This information was found to be of practical breeding value in Triticale. Similar information could be made use of in selecting strains for reduced amount of heterochromatin in umbelliferous species and to observe its effect on meiotic pairing.

(g) The fact that the heterochromatin segments are more susceptible to chromosome damaging effect of chemicals and radiations needs further exploration in relative terms as to whether particular class of heterochromatin is more easily affected. Studies on localised chromosome breakage effect by certain agents to attack only particular heterochromatic segments may be rewarding. Techniques are now available to provide fine details of different categories of heterochromatin according to the classification of Vosa[67,68] and also various other types of heterochromatic regions other than the one demonstrable by Giemsa and Q-banding technique *e.g.* Hy banding[69], O-banding[70].

(h) The role of heterochromatin in shortening the cell cycle duration has been suggested[56,57] but the data is limited to a selected number of plants. To further substantiate this role of heterochromatin, repetitive DNA and total DNA content in chromosomal evolution in relation to cell cycle duration and cell growth, information must be gathered from other sources too, particularly when data on nuclear DNA content are rapidly accumulating. An ideal material for such investigation may be *Lathyrus* in which information is available in related fields cited above[13,54,71,72].

(i) Whether the heterochromatin (particularly the procentric one) has some role in anaphase chromosome movements needs to be tested. Particular attention should be centred on heterochromatin recognition of cells where cytomixis or intraindividual chromosomal variability is very frequent and a large number of cells show variation in chromosome number in the same tissue of a single plant specially at ploidy level. The information is likely to provide a definite insight on the possible role of heterochromatin in chromosome movement.

(j) While discussing the nuclear DNA variation in plants[73,74] it has been suggested that during the phylogenetic evolution of species in a genus, usually the change in total DNA content is mainly due to its repetitive DNA fraction. The constitutive heterochromatin which reflects the repetitive DNA fraction may provide a substantial information at cytological level in this context. Studies on % heterochromatin of total chromatin, location of heterochromatic segments and their subsequent distribution pattern with regard to evolution may throw significant light on the role of heterochromatin in evolution and adaptability.

(k) In *Nicotiana* hybrids exhibiting hybrid induced changes in chromosome morphology, evidences suggest that the ability to produce megachromosomes in *N. tabacum*, is the general property of heterochromatin introduced into an alien background. As yet the mechanism of megachromosome production is unresolved. The investigators favour the hypothesis that physical proneness to breakage may in turn lead to the broken heterochromatin continuing replication beyond one cycle, hence to megachromosome formation[35]. It would be desirable to evaluate this hypothesis by analysing the interspecific hybrids of related species widely differing in their heterochromatin content.

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