Prof. Chandrasekhar Chakrabarti
Former Vice Chancellor'
WB University of Animal Husbandry & Fishery Sciences;
Former Faculty,
Burdwan University &
North Bengal University
cchakrabarti90@gmail.com

Heterochromatin and its Significance: A Review

Chandrasekhar Chakrabarti

Abstract:

All living organisms excluding virus, possess chromosome(S). Among eukaryotes the chromosomes are situated inside the nucleus number of chromosomes are variable among the organisms but fixed for a particular species. The structure of chromosome changes along with the phases of the Cell Cycle. In the interphase stage the chromosomes are very long and not distinctly countable. During metaphase stage the chromosomes take definite size and shape.At this stage, properly stained chromosomes can be counted even under the light compound microscope. The Chromosomes are formed by the combination of DNA and histone proteins. The DNA-protein combined structure is termed as the Chromatin. The density of the chromatin is not uniform. In some region the chromatin takes deep stain and, in some region, takes light stain. The deeply stained parts are called Heterochromatin and the lightly stained parts are called Euchromatin. In a typical cell in interphase, approximately 10% of the genome is packed into heterochromatin. The Heterochromatin is classified into facultative and constitutive. The constitutive heterochromatins are present mainly In the centromere/chromocenter and telomere areas of the chromosomes and play very important role in chromosome organization and cell division. Polytene chromosomes of different species of Drosophila have been studied to understand the significance of heterochromatin in the chromosome.

Key words: *polytene chromosome, heterochromatin, chromocentre,*

1. Introduction:

Emil Heitz(1928)[1] a co-discoverer of giant chromosome of diptera first coined the term heterochromatin. The Chromocentre of the polytene chromosome of *Drosophila* sp possess heterochromatin. This region is very important for maintaining the chromosome organization. Polytene chromosomes of *Drosophila* remains permanently at the interphase stage and maintain integrity during endoreduplication.

[1]Heitz,E.(1934) Uber alpha and beta –heterochomatin Sowie konstranz und Bau der chromomeren bei *Drosophila.* Bio.Zbl. 54 : 588-609

Chakrabarti(1975, 1990, 1991) [2] reported remarkable changes of the heterochromatic chromocentres and dark bands of polytene chromosomes of various species of *Drosophila* after trypsinization and fluorescent staining.

Before that Hilwig and Gropp(1972)[3] studied the constitutive hrterochromatin in mammalian genome with bibenzimidazole compound (Hoechst 33258). Applying a fluorescent dye Acridine orange (A O) reverse- banding method (Borrow et al,1972) [4] detected chromocentric heterochromatin in Bovidae. Schnedl(1972,1973) [5] studied Giemsa banding, quinacrine fluorescence and DNA replication in chromosomes of cattle(*Bos taurus).* Schnedl and Czaker(1974)[6] studied the chromocentric heterochromatin and compared G-banding in cattle, goat, and sheep chromosomes. Holmquist(1975)[7], Wheeler and Altenberg(1977), have studied polytene chromosomes of *Drosophila* sp. with Hoechst 33258 fluorescent staining.

Similarly, Hearly et, al. (1990)[8] and Hastie et al, (1990)[9] reported that the constitutive heterochomatin of the telomeres which form the terminal cap of the linear chromosomes are essential determinants of replicative potential and cellular ageing.

2. Methodology:

Different investigators used different organisms and methods in their experiments to identify the importance of heterochromatin. Chakrabarti (1975,78, 90, 91, 92 93a, 93b)[10] used polytene chromosomes of different species of *Drosophila* for various experiments. Salivary glands of third instar *Drosophila* larvae collected from the culture bottle were dissected out under the binoculars. Contralateral glands were incubated in Ringer buffer solution for controls and in various concentrations of trypsin solutions as per protocols. Squash preparations were done on clean grease free slides. Squash preparations were stained by various chromosome specific stains, and /or stained by the fluorochromes Hoechst 33258 or Acridine orange, as the case may

[2] Chakrabarti, C.S.; (1990) Effect of trypsin on the chromocentric mass of the salivary gland chromosomes of four species of *Drosophila.* Biol. Bull. India 12 :33-38.
[3] Hilwig, I. & Gropp, A.(1972). Staining of constitutive heterochromatin in mammalian chromosomes with a new fluorochrome. Exp. Cell Res.75:122-126.
[4] Borrow, M.; Collacott, H.E.A.C. & Madan, K.:(1972) Chromosome bandiung with acridine orange. Lancet ii :1311
[5] Schnedl, W. (1971a) Banding patterns of human chromosomes. Nature New Biol.233 : 93-94
[6] Schnedl, W., and Czaker, R. (1974) Centromeric heterochromatin and comparison of G-banding in cattle,goat,and sheep chromosomes(Bovidae), Cytogenet Cell Genet. 13 :246-255
[7] Holmquist, G.(1975) Hoechst 33258 fluorescent staining of *Drosophila* chromosomes. Chromosoma (Berl.)49 :339-356.
[8]Harley, C.B, Futcher, A.B., and Grieder,C.W.(1990) Telomeres shorten during ageing of human fibroblasts. Nature . 345 :458-460
[9] Hastie, N.D., Dempster, M,; Dunlop, ,M.G., Thomson, A.M., Green, D. k. & Allshire, R.C. (1990). Telomere reduction in human cholorectal carcinoma and with ageing. Nature.346 :866-868.
[10] Chakrabarti, C.S.:(1975). Effect of trypsin on banding and puffing pattern of *Drosophila* polytene chromosomes. M. Sc. Dissertation, Calcutta University.

be. Slides were observed under suitable compound microscopes under oil immersion objectives and photographs were taken for some experiments. In case of flourochrome staining experiments, the slides were observed under fluorescence microscope (Carl Zeiss Jena) with suitable filters and the photographs were taken by the camera attached with the microscope. Schnedl and Czaker established fibroblast cultures from cattle, domestic goats and domestic sheep and the chromosomes were prepared by air dry method following technique of Schnedl(1971a,1971b)[11] and trypsin method of Sperling and Weisner(1972). C banding of the chromosomes were done following Arrighi and Hsu (1971)[12] and Yunis et al,(1971)[13].

3. Observations and Discussions:

From the studies of Chakrabari and Mukherjee (1975,78), Chakrabarti(1990,91,92) it was clearly evident that trypsin can induce puffing activities of various bands of the male X chromosome of *Drosophila melanogaster* (See figs a, b, and c of plate 1). Moreover, Trypsin can produce distinct separation of alpha and beta heterochromatin in the chromocentre of *Drosophila melanogaster.* (see figs a, b, c of plate 2).

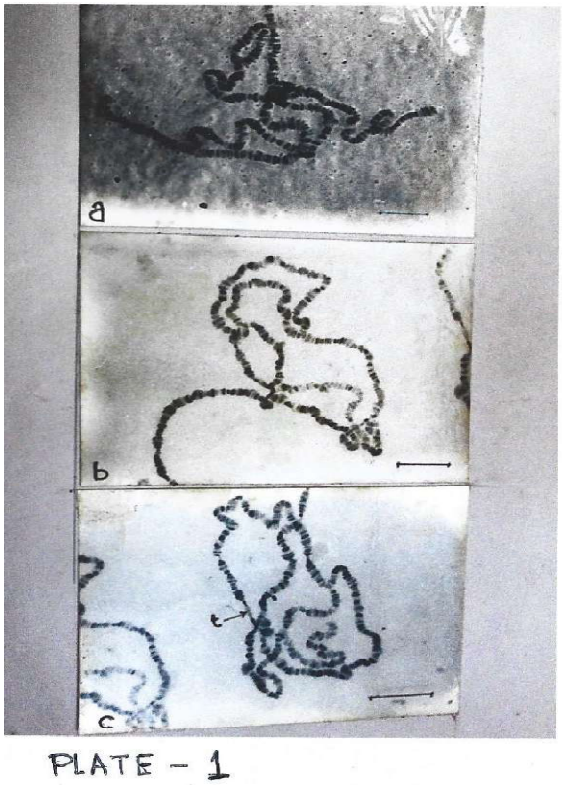

PLATE I : Control and Trypsin treated salivary gland Chromosomes of *Drosophila hydei.*

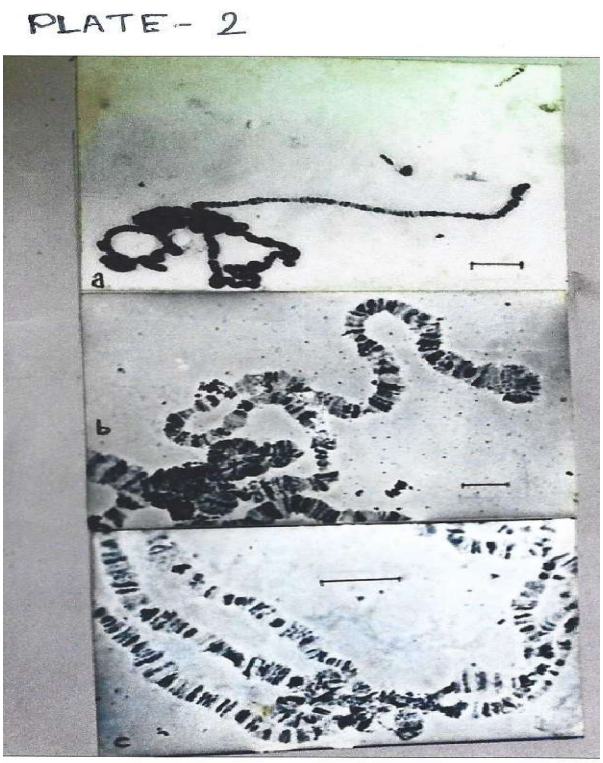

PLATE 2: Control and treated salivary gland chromosomes of *Drosophila melanogaster*

[11] Schnedl, W. (1971a). Banding pattern of human chromosomes. Nature New Biol. 233: 93-94.
[12] Arrighi, F.E & Hsu, T.C.:(1971) Localization of heterochromatin in human chromosomes. Cytogenetics 10:81-86.
[13] Yunis, J.J. & Yasmineh, W.G. (1971) Heterochromatin, satellite DNA, and cell function. Science 174: 1200-1209.

a) Control (without trypsin treatment). b) 0.10mg/ ml trypsin treated (showing broken chromocentre. c) 0.15 mg/ml trypsin treated (showing thread like structure (t) in the chromocentre. Bar =10µ

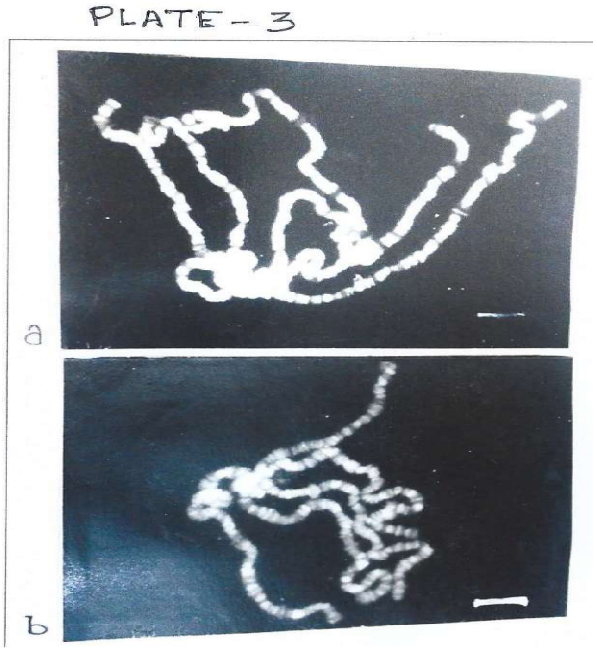

PLATE 3: Hochst 33258 Stained chromosomes of *Drosophila hydei*

a) Without trypsin treatment: Brightly fluorescent chromosome b) 0.05 mg/ml trypsin treated Hoechst33258 stained chromosome showing dull fluorescence. Bar =10µ

a) Control (without trypsin treatment),showing entire X chromosome. b) 0.10 mg/ml trypsin treated chromosome, showing puffing activities at various bands of the X chromosome. c) 0.15mg/ml trypsin treated chromosome showing disorganized chromocentre. Bar=10µ

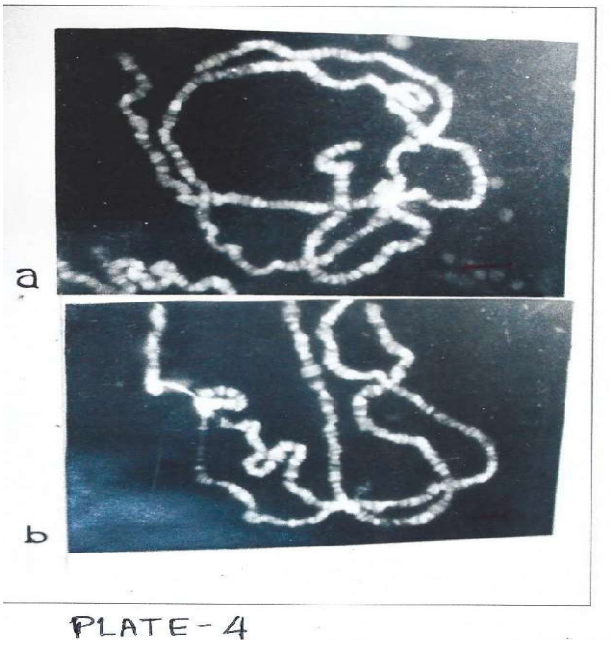

PLATE 4: Hoechst 33258 stained polytene chromosome.

a) 0.010 mg/ml trypsin treated Hoechst-stained chromosome showing variable fluorescence. b) 0.15mg/ml trypsin treated Hoechst-stained chromosome, showing variable fluorescence with reduced chromocenter. Bar =10µ

Findings of Chakrabarti and Mukherjee (1978)[14], Chakrabarti (1990)[15], supported the earlier findings of Sea Bright(1972) [16], Wang and Pedroff(1972) [17], Hirahara et al,(1973) [18]. Heterochromatin organization was further elucidated by fluorochrome staining by Schnedl and

[14] Chakrabarti, C.S., and Mukherjee ,A.S.(1978) Morphology and replication of trypsinized polytene chromosomes of *Drosophila.* In: Abstracts of Cell Biology Conference, Delhi University. Delhi.pp 41.

[15] Chakrabarti, C. S. (1990) Effect of trypsin on the chromocentric mass of the salivary gland chromosomes of four species of *Dros*ophila. Biol.Bull.India.12 : 33-38.

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Czaker(1974)[19], Chakrabarti(1993a,1993b)[20]. Hoechst 33258 stained non-trypsinized polytene chromosomes showed brighter fluorescence at the chromocenter and many dark bands, from this it has been suggested that this fluorochrome has special affinity with the heterochromatic regions of the polytene organization. *(see figs a, b of plate 3 and fig a, b of Plate- 4).* On the other hand, Acridine Orange stained trypsin treated chromosomes showed brighter fluoresce. Chakrabarti(1993b)[21].

The contrasting action of the two fluorochromes suggest that the Hoechst 33258 has special affinity to heterochomatin, so it produces brighter fluorescence when the heteochomatin is not digested by the enzyme trypsin. Trypsin causes unmasking of the DNA/Protein association of the chromocentre and at the dark bands; as a result, trypsin treated Hoechst 33258 stained show dull fluorescence. Earlier observations of Weisblum &Haenssler(1974)[22], Commings(1975)[23], Ghosh and Mukherjee(1982)[24] also showed special affinity of Hoechst 33258 to constitutive heterochomatin This affinity might be due to the fact that chromocentric heterchromatin is composed of AT nucleotides combined with some specific proteins,a unique H3 variant (Karp,2008)[25]. Possibly for this reason Hoechst 33258 showed special affinity to this compound present at the chromocenter. Karp(2008) in his book cited that at the centromeres of human chromosomes there are tandemly repeated 171 base pairs DNA sequence, termed as the alpha – satellite DNA. Allshire and Karper (2008)[26] showed that not only the repetitive DNA is required for the construction of heterochromatin near the centromere, RNAi regulatory proteins are also necessary for assembly and maintenance of silent chromatin in yeast.

Duggan & Tang(2010)[27] has reported that transcriptionally inactive heterochromatin plays a vital role in stabilizing the centromeres and the telomeres of the chromosomes. On the other hand, trypsin digested AO stained polytene chromosomes showed distinct greenish-yellowish

[19] Schnedl, W. & Czaker, R.(1974). Centromeric heterochromatin and comparison of G-banding in cattle, goat and sheep chromosomes (Bovidae). Cytogenet. Cell. Genet. 13: 246-255.
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fluorescence. The degree of brightness depends with the degree of trypsin digestion. This may be due to the fact that AO being an intercalating substance it binds with the nucleotides of the heterochromatin to produce bright fluorescence. This result also supports the earlier observations of Walle & Gorge (1989)[28], Tinwell & Ashby (1989)[29].

Like the centromeres, telomeres also maintain genomic integrity in normal cells, and their progressive shortening during successive cell divisions induces chromosomal instability. Telomere length is maintained by the enzyme telomerase. Therefore, maintenance of the length and the activity of telomerase are very crucial for the initiation and stability of cancer (MA Jafri et al, 2016)[30]. Wang J. et al, (2018)[31] reported that the telomeres convert adjacent chromatin into heterochromatin.

4. Comments:

From the information cited in this article it has become clear that heterochromatin is very important not only for the structural organization of the chromosome but plays a vital role in position effect variegation (PEV), that has become evident in case of white eye locus of *Drosophila.* PEV is developed due to spreading of heterochromatin package across the heterochromatin/euchromatin border that cause transcriptional silencing (Sarah and Reuter, 2013)[32]. Heterochromatin domains also play important role in chromosome organization, cell division, development and ageing, mammalian X chromosome inactivation and senescence (Wang J et al,2018). Trypsin treated fluorochrome stained studies on the polytene chromosomes has established a new dimension to the structural analysis of chromosomes, especially the constitutive heterochromatin of *Drosophila.*

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