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Research Article

NUCLEAR ARCHITECTURES OF CHROMOSOMES IN GERMINAL AND SOMATIC NUCLEI OF *MUS MUSCULUS* OF MANIPUR

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ABSTRACT
A numerous works on chromosome compartmentalization of chromosomes had been done in recent years using various organisms. Such works had been limited for *Mus* species. *Mus* species in most of them, with diploid number of 40 with all acrocentrics chromosomes also narrow down the study. Taking orientation of centromeric heterochromatins inside the nucleus as reference points, compartments of chromosomes inside the nuclei are studied. Germinal cells are harvested from testicular cells and somatic cells from bone marrow cells by standard hypotonic-air drying method. There are consistent four distinct compartments in the prophase I stages- leptotene, zygotene, pachytene, diplotene, diakinesis. At metaphase I, the smaller and longer chromosomes are separated into roughly four small compartments. In somatic interphase as well as in metaphase the compartmentalization is much similar to the germinal cells. Such study can be applied in phylogenetic studies on *Mus* species in future studies.

Keywords*: Compartmentalization, Germinal, Somatic Cells, Manipur*

INTRODUCTION
The cytogenetics in recent years, focus on the chromosome architecture than the number, structure and abnormalities in both plants and animals. Since the late 19th century, an uncounted number of microscopic studies have appeared on numerous aspects of nuclear structure and on the observation of mitotic chromosomes (Creamer and Creamer, 2010). A territorial organization of interphase chromosomes was first suggested for animal cell nuclei by Rabl (1885), but it was Theodor Boveri (1909) who introduced the term chromosome territory (CT) in his seminal studies of blastomere stages of the horse roundworm *Parascaris* or *Ascaris megalocephala*. Recent experiments concerning the radial positioning of chromosomes in the nuclear volume of human and primate lymphocyte cell suggest a relationship between the gene density of a chromosome territory (CT) and its distance to the nuclear centre (Kreth *et al.*, 2004). The simplest reason to organize chromosomes into domains in the nucleus is to prevent them from becoming entangled (Essers *et al.*, 2005). To decide about the random or non-random distribution of a given target, such as a CT, chromosomal sub region, or gene, it is important to define a simple or sometimes multiple 3D (three dimensional) reference points, which represent the target in question in the nuclear space. For a painted CT, its intensity gravity centre can be chosen as a single reference point or the CT surface may be used to define multiple reference points. Next, proper reference structures must be defined to decide whether the chosen target is distributed randomly or non-randomly with respect to them. For reference structures, one can choose other chromatin targets or distinct nuclear structures, e.g., the nuclear lamina, nucleoli, or splicing speckles (Ronneberger *et al.*, 2008).
For the first time *Mus* musculus castaneus germinal cells are analysed to know little about architecture in this genus despite lack of the sophisticated techniques and answer the query, do mice cells also have a unique nuclear architecture. There are consistent of four distinct compartments in the prophase I stages - leptotene, zygotene, pachytene, diplotene, diakinesis. At metaphase I, the smaller and longer chromosomes are separated into roughly four small compartments. In somatic interphase as well as in metaphase the compartmentalization is much similar to the germinal cells.

MATERIALS AND METHODS
Five male lived specimens of *Mus musculus castaneus* constituted the materials for the study. The identities of the mice were in accordance of Marshall, 1977. The animal was killed by inhalation of

chloroform in a close chamber and the testes were removed immediately. For germinal cell study testes were used and for somatic chromosome study bone marrow cells were taken. The tunica albuginia of each testis was removed; seminiferous tubules were put in 1 ml of RPMI 1460 (Himedia) in a cavity block and chopped thoroughly with a pair of sharp scissors. The suspension was left undisturbed for 20 minutes. The supernatant was transferred to a centrifuge tube with a Pasteur pipette and then centrifuged at 2600 rpm for 10 minutes. The supernatant was discarded and cell pellet was exposed to 76% KCl solution for 20 minutes. Then the tube was centrifuged at 800 rpm for 5 minutes. The supernatant was discarded and the residue was fixed in freshly prepared 1:3 acetic acid methanol for 20 minutes and spread on clean pre-chilled slides and dry. The slides were kept in desiccators for dehydration. Slides were stained in 4% Giemsa stained for 10 minutes and observed under 100X in Olympus microscope that was having 7.2 megapixel digital camera on it.

The chromosomes were stained for C-banding following the method of Sumner (1972) with slight modification. Seven days old slides were treated with 0.2MHCl (6.18ml/l) for 45 min. at room temperature and saturated Barium hydroxide solution (5%) for 9 minutes at 600C in a water bath, and thoroughly rinsed with distilled water. The barium hydroxide treated slides were then transferred to 2×SSC (880gm of sodium chloride and 440gm of sodium citrate in 50ml distilled water) solution for 1 hour at 680C in water bath. The slides were rinsed thoroughly with distilled water and dried. The dried pre-treated slides were stained in 6% sodium phosphate buffered Giemsa stain at pH 6.8 for 20 min. Finally, the slides were rinsed with Xylene for 40 sec. and mounted in DPX. At least 100 cells of each stages were recorded and taken 10 well distinguishable cells photographs of each stages.

RESULTS AND DISCUSSION

The germinal cells are conservative in nature as compared to somatic cells concerning the nuclear architecture so they are good option for studying the nuclear architectures with the reference points of centromeres in the chromosomes through C-band techniques. In *Mus musculus castaneus*, as a representative of *Mus* genus, the interphase is manifested with a deeply stain in C-banded nucleus and un-staining uniform spherical mass. Such deeply stained body is segregated into respective centromeres of the chromosomes (Figure 1. A). The C-band positive centromeres are arranged in a specific non-random manner inside the nucleus in somatic cells also (Figure 1.B). They are arranged into four distinct domains starting from interphase stages (Figure 1 C, D) and in leptotene stages (Figure 1. E, F). At the beginning of zygotene stage the synapsis is performed inside the specific domains of the respective bivalents (Figure 1.G, H, I). In the pachytene stage the tetravalent are somewhat disturbed in respect to the non-random arrangement but still maintained the four domains (Figure 1.J, K, L). The diplotene stage the segregation of the tetravalent are initiated by longer ones and occurred inside the respective domains of four (Figure 1. M). There is congregation of the chromosomes into four domains but distinct in compared to the preceding stages (Figure 1.N). At the metaphase I, the chromosomes are arranged in a circular manner in which the longer and smaller chromosomes are easily differentiated, in other word they are not overlap, but maintain the four domain arrangement (Figure 1.O, P). The arrangement of circular pattern specific to Metaphase stage is maintained in the Metaphase stage II (Figure 1.Q). In the remaining stages –anaphase I, II and telophases, the exact arrangement is not confirmed due to unable to observe under microscope that might be due very short cycle period in the *Mus*.

What factors deicide the nuclear architecture and when the phenomenon starts are common queries that await for answers. Recently it has been demonstrated that these chromosome bands are maintained in interphase nuclei as focal chromatin aggregations (Sadoni *et al.*, 1999) built up by a number of chromatin domains in the order of ~1Mb. These domains apparently persist through all interphase stages, show distinct nuclear localization patterns, and may provide an important component of the higher order nuclear architecture (Cremer and Cremer 2001). The present results are at early stage to come to a decisive conclusion, more species and more cells to be analysed from different populations and habitats with much advanced techniques. The results also support the Rabl arrangement of the chromosomes inside the nucleus

It can be concluded that there are four domains in germinal cells of mice nuclei that are visible to the conventional C-band method and it will be of interesting to compare the results with other species of the same genus or different genera. The architecture is limited to some cells and need intensive study but with much sophisticated devices the features will be quite different in future.

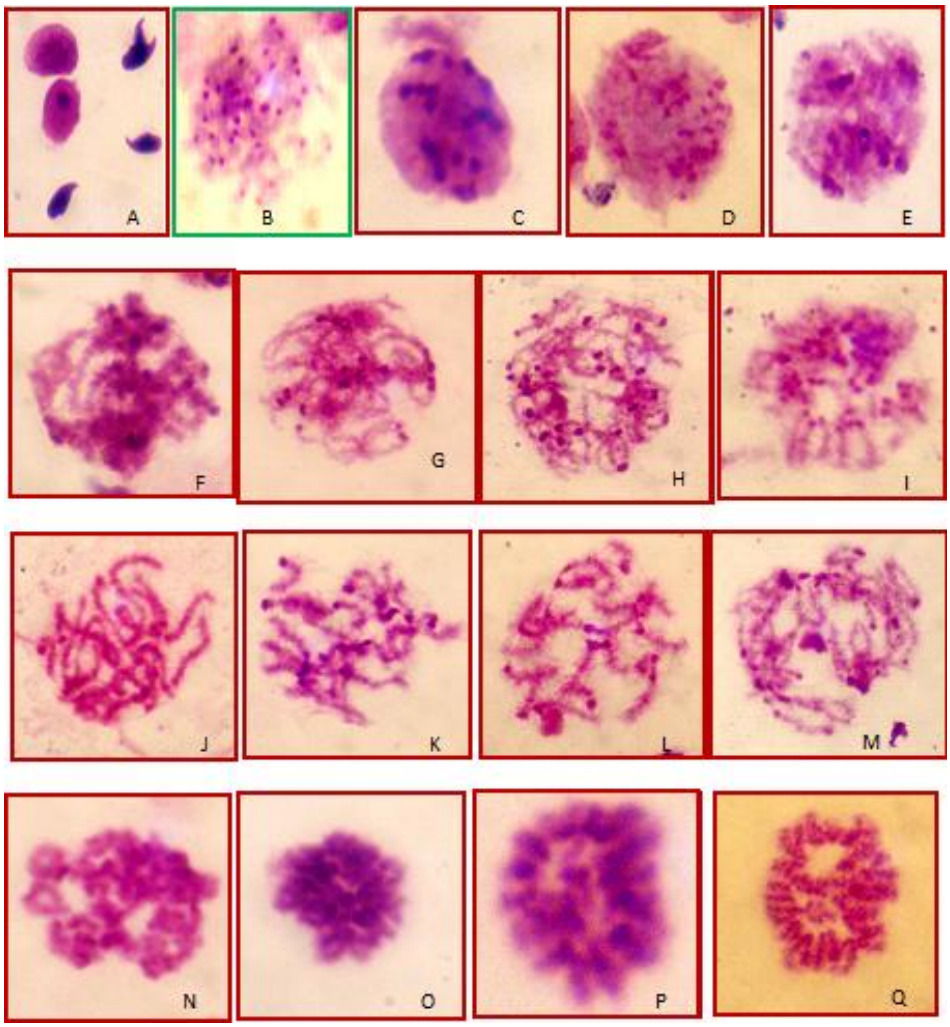

Figure 1: Germinal cell cycle in *Mus musculus castaneus*, A- Interphase nuclei with sperm head, B-Somatic C-banded chromosomes, C-Non random arrangement of chromocenters in four sites inside the nuclei, D-Early leptotene with centromeres into specific pattern, E-mid leptotene organized in four domains, F- Late leptotene with four domains, G-Early zygotene with bivalents showing synapsis but retain the four domains, H-Mid zygotene retaining the domains, I-Late zygotene also retained the domains, J-Early pachytene also roughly the four domains, K- Mid pachytene maintaining the domains, L-Late pachytene maintain the four domains and the sex chromatin is at the periphery, M- Diplotene cell shows roughly four domains, N- , O-Diakenesis showing congregations of tetravalents in four domains, P-Metaphase I the chromosomes are arranged in a circular manner into four domains, Q-Metaphase II arrangement is just like that of Metaphase I.

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