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Nucleogenesis during microsporogenesis of *Rheo discolor*

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ABSTRACT

The present paper deals with the study of Nucleogenesis and nucleolar behavior during microsporogenesis of Rheo discolor using silver- nucleolar organizer regions (NOR) staining technique. It is observed that only one nucleolar organizer regions is present in each genome of R. discolor and NORs are achieved during the Pre-meiotic and meiotic stages. It is observed that common pattern of nucleolar activity is not conserved in the present plants and process of nucleogenesis is similar in both mitotic and meiotic cell divisions.

Keywords: *R. discolor*, nucleogenesis, Ag-NOR

INTRODUCTION

Nucleogenesis, the formation of the nucleolus, is a very dynamic process in the fundamental life of the cells. It has been studied exclusively during the mitotic cell cycle using light and electron microscopic silver staining and immunoflorescence techniques [1]. During mitosis, the reformation of the nucleolus begins in late anaphase when nucleolar material appears in the vicinity and on the surface of the chromosomes, sometimes in the form of perichromosomal sheath. This nucleolus like material then condenses or collects into discrete structures termed perinucleolar bodies (PNBs) which subsequently fuse at the chromosomal nucleolar organizer regions (NORs) in telophase stage and early interphase nucleolus. PNBs have been described as dense circular fibrogranular structures [2], which contain RNA and protein which found in micronuclei [3-4] which stain with silver [5-9].

Nucleolus is a well defined domain of the interphase cell nucleus where ribosomal RNA (rRNA) is synthesized. They are also highly ordered nuclear component which provide an opportunity to study the mode of integration of several complex functions within restricted nuclear territories. They are sites of ribosome biogenesis, which involves transcription of rRNA genes, association of nascent transcripts with specific proteins and multiple steps of maturation of primary transcripts leading to the formation of the pre-ribosomal subunits. The main and permanent constituents of the nuclear body are the dense fibrilar and granular ribonucleoprotein components. It is generally agreed that in the nucleolus, ribosomal genes are located in the fibrilar centres and associated dense fibrilar component [10-12]. In these fibrilar structures, everything necessary for transcription is present.

Silver-Staining patterns of NORs in nuclei and chromosomes in pre- and post- meiotic stages of spermatogenesis have been observed in ten different species of vertebrates [13-14]. Similarly, Silver-Staining patterns were observed during spermatogenesis of all the species. Positive silver staining was evident in spermatogonia and throughout meiotic prophase, although silver staining was totally absent during metaphase I and metaphase II of meiosis. Ag-NORs reappeared in early spermatids, suggesting a post meiotic reactivation of NORs. The Ag-NORs then disappeared at the beginning of the elongation phase of the spermatid nucleus. This result indicates that NORs are

suppressed during meiotic divisions but become reactivated in the haploid spermatid. Haploid gene expression has been suggested for other genes, and this probably occurs with the ribosomal gene.

The behavior of nucleolus during meiosis has been systematically analyzed in a few species of mammals [15] insect [16] and plant [17-18]. PNB-like structures have been observed in meiotic cells of *Locusta migratoria* [19] and *Allium flavum* [17]. The studies show the presence of nucleolus throughout prophase stages upto pachytene in vertebrates and upto metaphase I in insects and plants.

The above observations form the basis for interpretation of the findings on nucleogenesis and meiotic prophase I stages. However, process of nucleogenesis during meiosis has not been described in any species of both plants and animals. Consequently, the nature of relationship between NOR activity and its morphological consequences, formation of nucleolus has not been elucidated.

Light microscopic analysis of silver-stained PMCS of Tradiscantia *Rhoeo discolor* could relate stages of nucleogenesis to pre-meiotic and meiotic prophase I permitting observation of meiotic cell stages with respect to nucleolar formation.

MATERIALS AND METHODS

Tradescantia *Rhoeo discolor* plants were grown in pots under natural conditions. Flowering takes place throughout the year, peak timing of flowering falls during February to June. Flowers at different sizes were collected from the inflorescence during 8-10 hours in the morning. Dividing stages of the pollen mother cells were determined by acetocarmine squash technique. Sizes of the anthers having desired stages of cell division were measured using a calibrated ocular micrometer. Flowers selected visually were dissected out and anther size was measured. Meiotic stages were found in anthers of size range 20-40 ocular units in breath and 39-83 ocular units in length. Anthers of little smaller size provided premeiotic stages.

Flowers having appropriate anther sizes were selected and fixed in methanol-chloroform-acetic acid (6:3:2) for overnight inside the freezing chamber of refrigerator. The Pollen Mother Cells (PMCs) were squeezed out of the anthers in a drop of 45% acetic acid on a clean slide and squashed under a coverslip after warming the slide over flame. Then the slides were kept in refrigerated dessicator at 0^{o}C for at least one day or till further process. The cover slip was flipped off with a needle and the preparations were allowed to air-dried. Refrigerated dessication made the cells to stick to the slide saving the cells from lost with coverslip. The slides were stained with silver nitrate following the method of [20] modified by [21].

The method is as follows- Colloidal developer was prepared by mixing 2g of powdered gelatin in 100 ml of distilled water and 1 ml of pure formic acid. Gelatin was dissolved by slightly warming the solution for a few minutes at 37^{o}C. 50% silver nitrate solution was prepared by dissolving 4g of Ag-NO_3 in 8 ml of distilled water. Two drops of colloidal developer and four drops of aqueous silver nitrate were pipette onto the slides. A coverslip was put and the slide was placed for 2 minutes on a slide warmer pre-heated at 68-70^{o}C for 2 minutes on a slide warmer. The slide was immersed in running distilled water, the coverslip was removed and rinsed for 10 minutes, air dried, soaked in xylene and mounted in DPX. The slides were examined under bright field optics of Leitz compound light microscope. The selected cells were photographed with 100x objective using Agfa orthopan 25 ASA black and white films. The meiotic stages were identified following the classical stages of meiosis.

RESULTS

Analysis of silver-stained PMCs of *Rhoeo discolor* reveals that silver nitrate stains nucleolar materials and nucleoli during premeiotic and first meiotic prophase upto pachytene. The nucleolar materials and nucleolus were referred to premeiotic and meiotic stages which were identified by chromatin morphology and behavior. The stages of nucleologenesis referred to premeiotic and meiotic stages are described below:

Preleptotene

The cells were characterized by complete absence of chromosomes and diffusion of chromatin throughout the nucleus. Several silver-stained spherical bodies of different sizes were present as shown in (Fig.1.1). These bodies correspond to the structures designated as the prenucleolar bodies (PNBs) described in somatic cells [5].

Early leptotene
Chromatin started condensation and organization into chromosomes appear as thin thread-like structures. The chromosomes were randomly dispersed throughout the nuclear area. Organization of PNBs into nucleolus began at this stage (Fig.1.2). Several PNBs aggregated at the NORs while several others remained scattered.

Late leptotene
More number of chromosomes was present. Fusion of PNBs into larger units took place and these larger bodies were in the presence of fusion into nucleolus (Fig. 1.3). Due to the fusion of PNBs into nucleolus, the number of PNBs decreased with the advancement of leptotene stage.

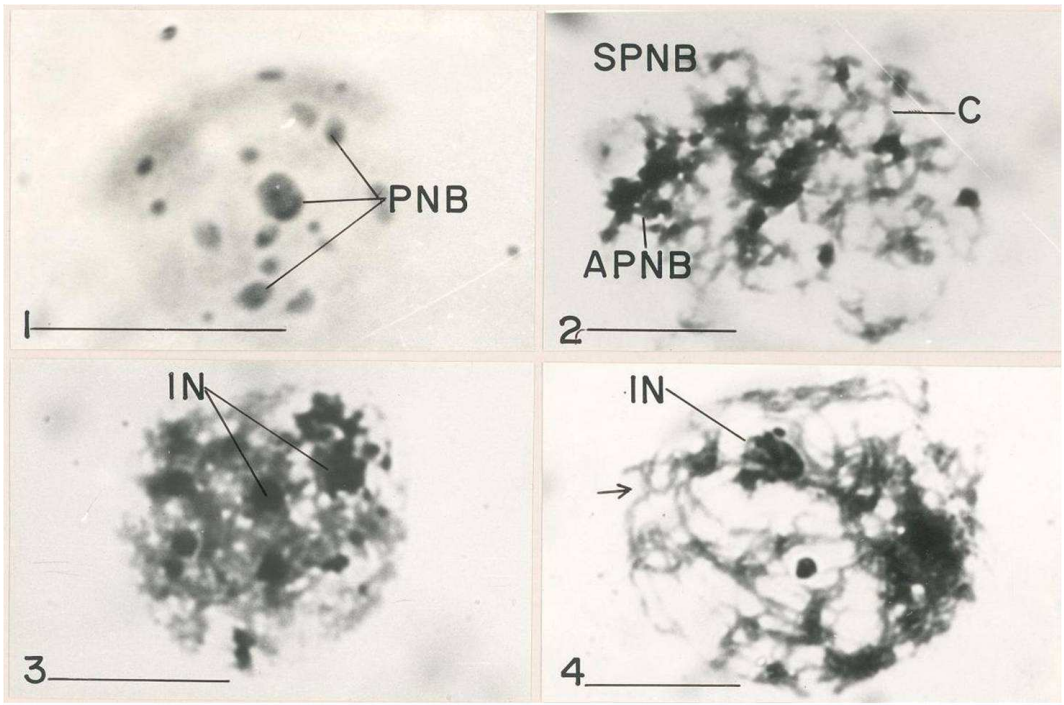

Fig. 1 Stages of nucleologenesis . 1. *Preleptotene, 2. Early leptotene, 3. Late leptotene, 4. Early zygotene*

Early zygotene
Early zygotene was characterized by the beginning of synapsis of homologous chromosomes. Many PNBs had fused into immature nucleoli (Fig. 1.4). Maximum number of nucleolar bodies (NBs) observed at this stage was four in number (Fig. 2.5).

Mid zygotene
With the advancement of synapsis, formation of nucleolus was also progressed. One mature nucleolus was found at the periphery, while the other NBs remain dissociated as shown in (Fig. 2.6). In zygotene cells more advanced than the former also, fusion of NBs had not still completed and only one nucleolus appeared at the periphery (Fig. 2.7).

Late zygotene
This stage was characterized by the advanced stage of synapsis. In each of the cells at this stage, two nucleoli of similar (Fig. 2.8) or dissimilar (Fig. 3.9) sizes and intensity of staining were invariably present. Each nucleolus was spherical in shape. The entire area was stained with silver except at different regions which were completely silver negative (Fig. 2.8 & Fig. 3.9, 3.10) very small zygotene nucleolus was usually stained with silver throughout the area without any unstained region (Fig.3. 9).

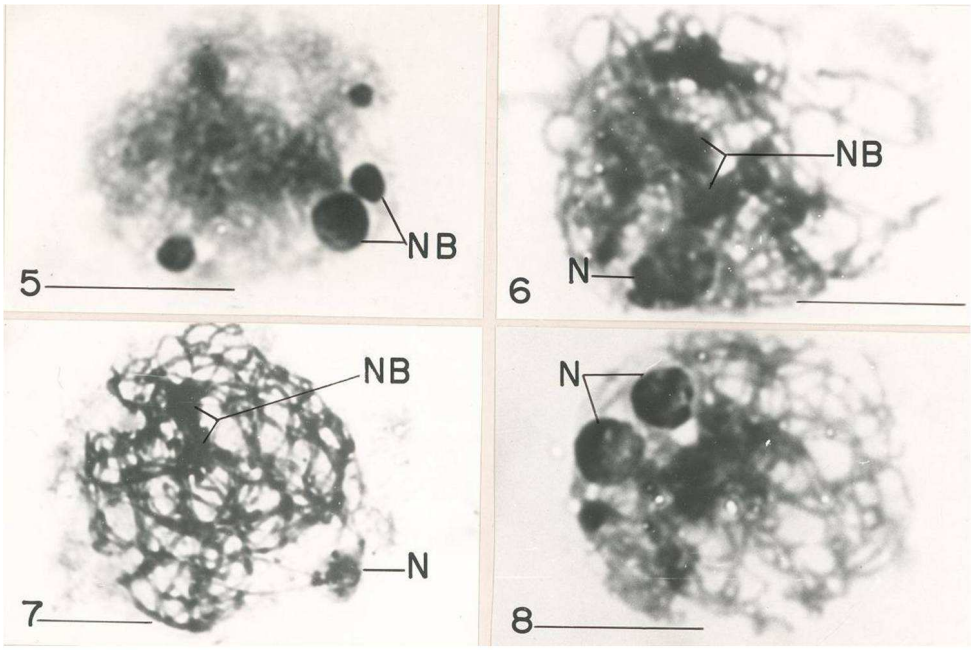

Fig. 2 Stages of nucleologenesis 5. *Early zygotene , 6. Mid zygotene , 7 & 8. Late zygotene*

Pachytene

Synapsis was completed and six bivalents were present in each cell. In most of the early pachytene cells, two nucleoli of different sizes were present and these nucleoli were smaller in size and lesser in density of staining than those of zygotene cells indicating the beginning of nucleolar degeneration (Fig. 3.10). In other pachytene nucleoli only remants of nucleolus were present (Fig. 3.11).

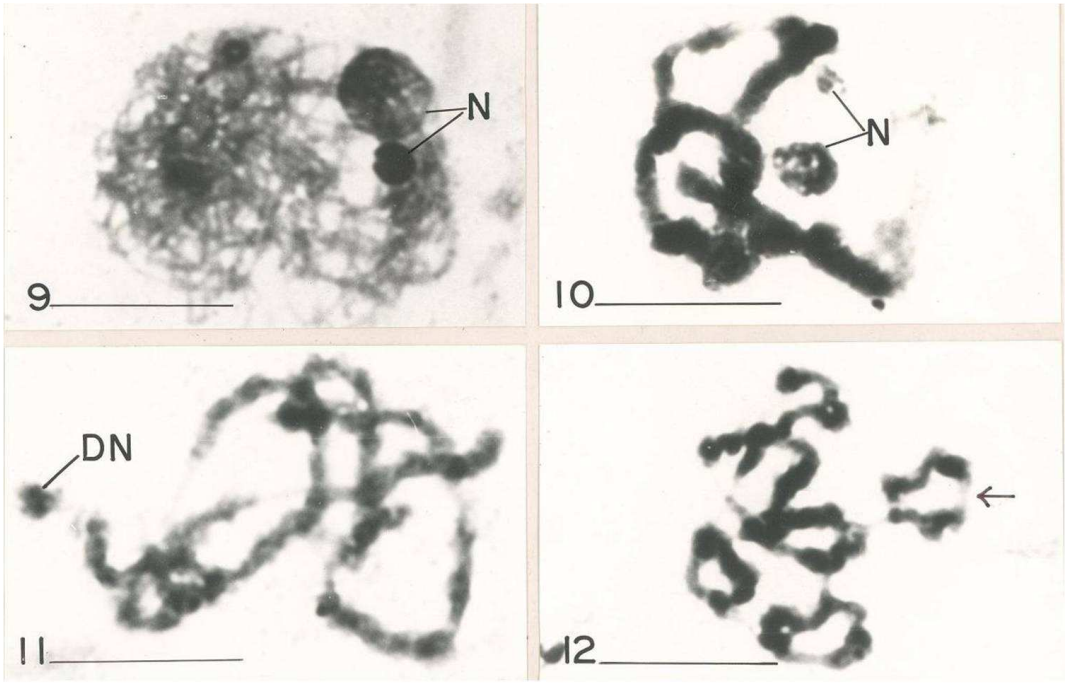

Fig. 3 Stages of nucleologenesis 9. *Late zygotene, 10. Pachytene, 11. Pachytene, 12. Diakinesis*

Diakinesis
Cells in diakinesis were characterized by disjunction of homologous chromosomes of each bivalent except at the telomeres. Nucleoli were completely absent from cells at this stage (Fig. 3. 12).

DISCUSSION

Although numerous meiotic studies have noted the occurrence of nucleoli during the early meiotic prophase there is no report of systematic studies on nucleogenesis and nucleolar behavior during meiosis. The only report on meiotic nucleolus behavior in plants is of [18] in wheat-rye hybrid. They observed that silver nitrate stains nucleoli during the first meiotic prophase until pachytene. However, in *R. discolor* plants analyzed here, silver stains nucleoli upto pacytene. It appears that a common pattern of nucleolar activity is not conserved in plants.

In each late zygotene cell, two pairs of nucleoli of similar (Fig.2. 8) or different (Fig.3. 9) sizes and degrees of staining were present. This suggests the presence of only one pair of NORs per genome of *R. discolor*. However, nucleoli may fuse into large nucleolus and consequently in later stages of cell division, probability of finding cells with actual number of nucleoli decreases drastically as observed by [18] in wheat-rye hybrid.

Nucleolar structures usually show differentiation in two components, pars fibrosa and pars granulosa in meiotic cells of *Allium cepa* anthers [7]. Loidl et al. [17] observed at the light microscopic level in meiotic cells of anther of *A. flavum* that from early leptotene onwards the nucleoli appear bipartite; a heavily silver impregnated core which appears black is surrounded by weakly stained brown shell. They further observed that during zygotene and pachytene each NOR chromosomes have its own core within the nucleolus. However, such structures, were not observed in the present study which revealed that each nucleolus was spherical in shape and the entire area was stained with silver except at various regions which were completely Ag-ve (Fig. 2.8 & 3.9, 3.10). Extension of Ag-NOR staining technique to nucleolar studies in various plants and animal species is required before any attempt is made to draw a conclusion on the degree of resolution of nucleolar structure at the light microscope level by silver staining technique.
It has also been known that NORs are active during the early meiotic prophase upto pachytene and the ribosomal genes are rendered inactive throughout the remaining stages of meiosis [14]. The results of the present study lend support to view as occurrence of nucleogenesis and nucleolar activity during pre-meiotic interphase and meiotic prophase I stages upto pachytene observed in the present study shows that NORs are active during pre-meiotic interphase and early meiotic prophase I stages. The transcriptional timing of rDNA inferred in the present study from the timing of nucleogenesis and nucleolar behavior does not correspond to the general pattern of Ag-NOR during meiosis observed in several species by [13-14]. So, these observation are also supportive evidences for the finding of [22-23] that Ag-NOR staining is rather associated with rDNA chromatin decondensation than transcriptional activity of ribosomal genes.

From similar studies on mitotic cell it has been established that rDNA containing NOR performs two functions: (a) synthesis of 18s and 25s RNAs and (b) organization of this RNA together with DNA, ribosomal and other nucleolar proteins into nucleolus [24]. Process of nucleogenesis in somatic cells, the morphological consequences of this biochemical process, includes appearance of perichromosomal sheath-like perinucleolar material, condensation of this material into nucleolus [1]. The same sequences of events were also observed in the present study (Fig. 1.1to 1.4 & 2.6). Obviously transcriptional pattern of rDNA in mitotic and meiotic chromosomes is similar.

PNBs contain RNAs and proteins [3-4] and the proteins have been identified as nucleolar phosphoprotein B_{23} and C_{23} [1]. The B_{23} is associated with RNA containing structure [25] providing evidence for the presence of RNA in PNB. Fusion of PNBs may not occur naturally [26], or may be prevented by actinomycin D [1], [8]&[27]. These observations demonstrate that PNBs are biochemically and functionally “miniature nucleoli”. It follows that transcription of NORs occurs during organization of PNBs which take place at premeiotic interphase and leptotene in *R. discolor* takes place at preleptotene and leptotene. This conclusion gains support from high resolution autoradiographic studies. Pattern of 3H uridine incorporation in dividing mouse Ehrlich tumour cells shows that NORs synthesize RNA for one part during the last G_2 of the previou cell cycle and for the other part during the telophase in which stages PNBs appear [28].

CONCLUSION

Nucleogenesis and nucleolar behavior have been studied during microsporogenesis of *Rhoeo discolor* using silver-NOR staining technique and light microscope. Several silver-stained spherical pre nucleolar bodies (PNBs) of different sizes were present in preleptotene nuclei. At early leptotene several PNBs aggregated at the NORs while several others remained scattered. Fusion of PNBs into nucleolus took place at late leptotene and the number of PNBs deceased with the advancement of leptotene stage. At early zygotene PNBs fused into four or more immature nuclei. Two mature nucleoli of similar or dissimilar sizes and intensities of staining were invariably present and two degenerating nucleoli were present. At diakinesis, nucleoli were completely absent. From this study the following conclusions have been drawn that only one NOR is present in each genome of *R. discolor*, NORs are active during pre-meiotic and meiotic stages, a common pattern of nucleolar activity is not conserved in plants, and transcriptional timing of rDNA does not correspond to the behavior of Ag-NOR, process of nucleogenesis is similar in both mitotic and meiotic cell divisions.

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