

ULTRASTRUCTURAL STUDY OF SYNAPTONEMAL COMPLEX IN MEIOCYTES OF *DATURA INNOXIA*

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Abstract

At the ultrastructural level Synaptonemal Complexes were studied in *Datura innoxia*, a polyploid. The attachment of the chromosome ends with the nuclear membrane is documented. In addition, our study has revealed the general features of synaptonemal complexes associated with centromeres (CM) and recombination nodules (RNs). The present paper also describes first time a close relationship between nucleolus and chromosomes.

Introduction

In the recent years, structures termed as Synaptonemal Complexes (SC) have been discovered in a number of organisms during meiosis. The SC is seen in various *Arthropods* such as *Culex*, *Anopheles*, *Locusta* and *Pollicipes* (Moses, 1968; Jaworska & Lima-de-Faria, 1969; Wettstein & Sotelo, 1971; Dudley, 1973). Also examples of SC in fungi and higher plants have been reported (Engels & Croes, 1968; Moens & Rapport, 1971; Gillies, 1981). In all these cases SC have been found after the pachytene of the meiotic cells. Further, since the discovery of synaptonemal complex in Cray fish numerous studies especially electron microscopic analysis have been made on the structure and function of chromosomal formulations. It is established now that the SC is found associated with homologous chromosome segments that are in meiotic prophase 1 (Denton *et al.*, 1976; Crosby-Longwell & Svihla, 1960; Givens & Phillips, 1976; Goodpasture & Bloom, 1975).

The nucleolus organizer region (NOR) is an area of the chromosome frequently characterized with secondary constriction (Heitz, 1931). It is defined as a region of chromosome containing the major rRNA genes; it is made up of chromatin fibrils. The nucleolar organizer regions were originally identified by Heitz in 1931. He showed the correlation between size and number of nuclei arising in telophase. McClintock (1934), however, working with translocation line in *Zea mays* argued against the hypothesis of Heitz, coining the term nucleolar organizer (NORs) to describe large knob of heterochromatin adjacent to the secondary constriction.

A number of ultrastructural studies have been carried out on pollens (Perveen & Qaiser, 2005) and in stomata types (Perveen *et al.*, 2007). Others linked the ultrastructural organization with function (Qadri *et al.*, 2008). Ultrastructural studies on nuclear organization and functioning in plant cells are scarce and mainly deal with species having reticulate type of nucleus (Risueno & Diaz de la Espina, 1979; Jordan *et al.*, 1980; Luck & Lafontaine, 1983), while studies on non-reticulate and chromomeric nuclei are very few (Deltour & Barsy 1985). The majority of studies deal with either

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chromatin organization (Barlow, 1977; Nagl, 1982), the nucleolus (Deltour & Barsy, 1985; Luck & Lafontaine, 1980; Motte *et al.*, 1988; Martin *et al.*, 1989), or nuclear bodies (Risueno *et al.*, 1978; Barlow, 1981, 1983a & b; Luck & Lafontaine, 1982; Williams *et al.*, 1985). The present paper describes the chromosomal formulations of homologous chromosomes associated with nucleolus, synapsis, chiasmata formation and crossing over at meiotic prophase.

Materials and Methods

The anthers were fixed in ice-cold 3-glutaraldehyde in 0.2M Phosphate buffer (pH 7) for 4-5 hours. The material was then washed and left over night in Phosphate buffer. Post fixation was carried out in 1% Osmium tetroxide for 2 hours at room temperature. After 3-4 washes with phosphate buffer, the material was dehydrated and infiltrated in series of increasing concentrations of ethanol and Epon resin. The material was then embedded in pure resin by using plastic embedding moulds. For polymerization, the moulds were placed in an incubator at 37 °C overnight followed by 60 °C for 48 hours. For electron microscopy ultra-thin sections of silver to pale gold color were cut with LKB-2088 ultramicrotome using diamond knife and were collected over copper grids. These were contrasted with Uranyl acetate followed by lead citrate (Reynolds, 1963). The observations were carried out with JX 100 Joel Transmission Electron Microscope.

Results and Discussion

Since the discovery of Synaptonemal Complex (SC) in Cray fish, numerous studies especially electron microscopic analysis have been made on the structure and function of these chromosomal formulations in meiotic prophase 1. There are also reports on ultrastructural studies on the sequence of events leading to Synaptonemal Complex formation initiated by Moens (1968). The process of degradation during diplotene remains relatively obscure. In allotriploid, Moens (1968) observed unusual tube-like structures over a distance of few micrometers area on Synaptonemal Complex elements at diplotene stage in plant meiotic cells. No such unusual structure was observed in our study. However, both the lateral and central elements of SC have same electron density and are less stained than the chromatin (Figs. 2 and 3, SCs). The centromere observed in this study accord well with previous descriptions from section preparations (Hasekamp, 1984; Heyting, *et al.*, 1988; Holm, 1986; Nokkala & Nokkala, 1985). The paired centromere appears as fuzzy, roughly spherical structures surrounding the SCs (Fig. 2, CM). Similarly the present ultramicrographs were remarkable in revealing clearly defined structures closely resembling bodies previously described in other species as recombination nodules (Figs. 3 and 4, RNs). These structures resembled the RNs described in *Drosophila melanogaster* and in *Zea mays* (Albini *et al.*, 1984; Westergaard & Wettstein, 1972).

Further in this investigation Synaptonemal Complexes were studied under polyploid condition with special reference to its relationship with the nucleolus. It was noted that the attachment of the chromosomes ends with the nuclear membrane, which is a unique situation in plants. In our study, not only the attachment, (Fig. 1) but also the recombination nodules (Fig. 2, 3RN) are documented for the first time in *Datura innoxii* as seen in earlier studies on *Rhoe* and *Chloeaaltus* (Moens, 1968) where at the end of the

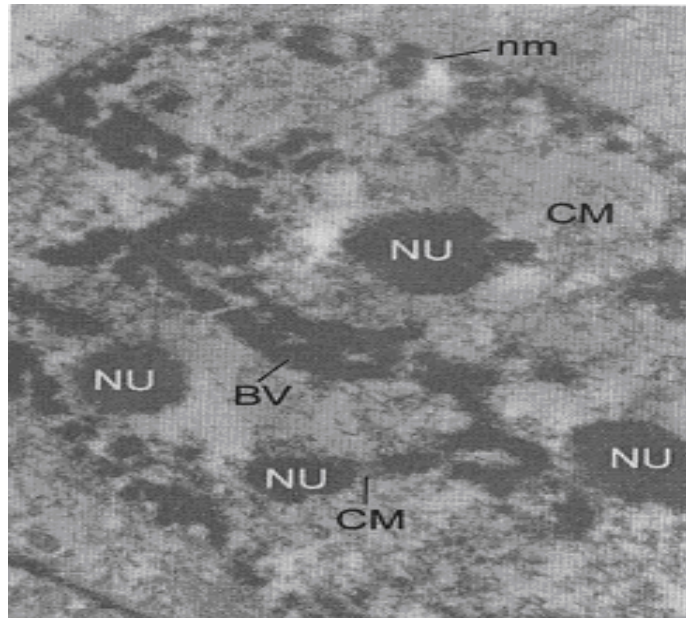


Fig. 1: Electron micrograph of meocytes showing 4-nucleoli (NU), Centromere (CM), and association of chromatin with nuclear membrane, X25000

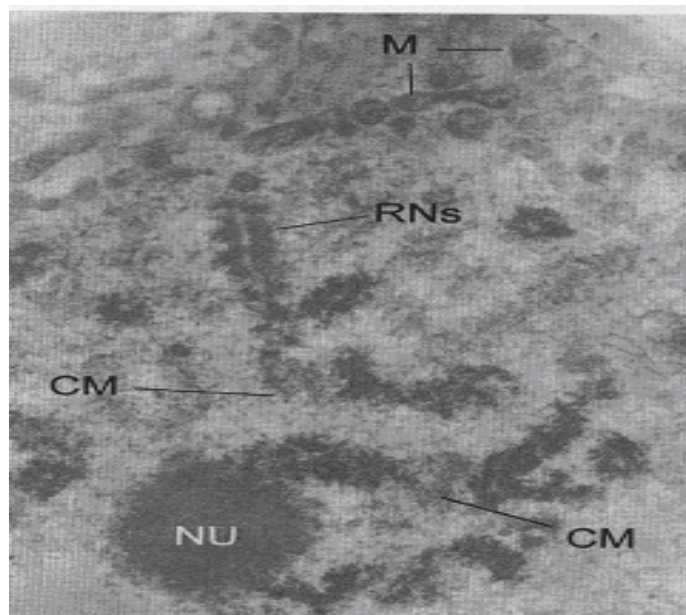


Fig. 2: A section of late Pachytene showing association of chromosome with nucleolus, which shows centromere (CM). Recombination nodules (RNs) are also evident in the adjacent sectioned bivalent (BV) Mitochondria (M), X28000

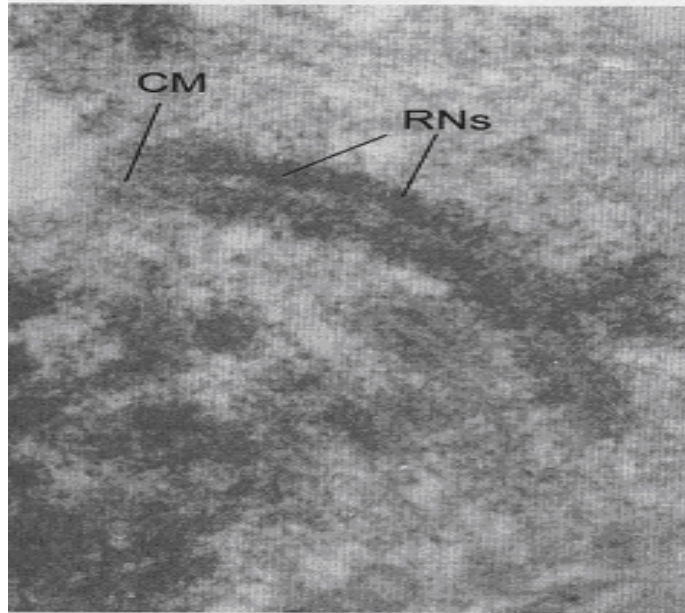


Fig. 3: A portion of Fig.2 enlarged, micrograph to show structure of Synaptonemal Complex marked with recombination nodules (RNs) and centromere (CM). X29000

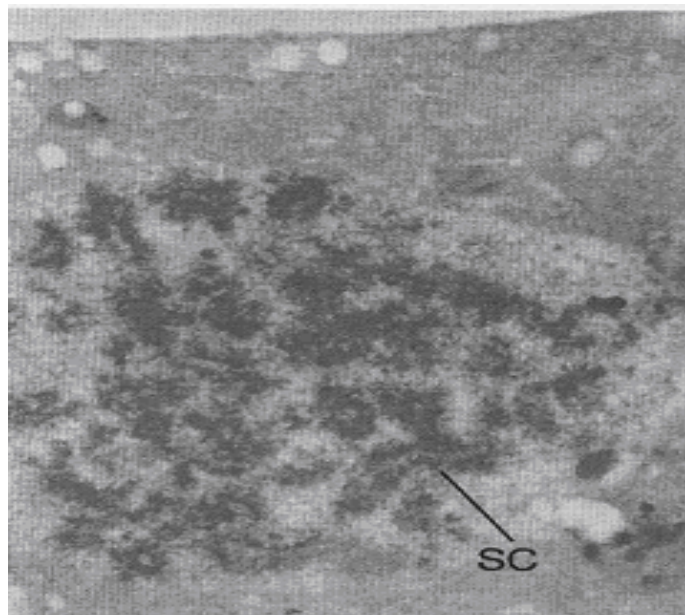


Fig. 4: Ultramicrograph shows meiocytes at Diakinesis, where the most of bivalent are sectioned transversely and some tangentially. At certain points centromere (CS) can also be identified X25000

synapsed bivalents the chromosome is seen associated with the nucleolus (Fig. 2, NU). This confirms the relationship between nucleolus and the chromosome. The chromosome emerging out of the nucleolus is also seen with chromocentre (Fig. 2, CM). Ultrastructural investigations over the last few decades, in a variety of both animal and plant cells, have disclosed the association of nucleolus with chromosomes of vegetative cells (Ashraf & Godward, 1980; Givens & Philips, 1976; Goodpasture & Bloom, 1975; Maggini *et al.*, 1978), animals and fungi (Jimenez-Garcia *et al.*, 1989). Further, this data supports the view of a close morphological and functional similarity in the organization of the nucleoli of multicellular eukaryotes (Barlow, 1977; Nagl, 1985; Church & Moens 1976; Lafontaine *et al.*, 1979). The chromocentres are also called "micropuffs" in reticulate nucleolar plant species (Risueno *et al.*, 1978), which are seen associated with nucleolus in meiotic prophase 1 (Lafontain & Luck, 1980). A similar situation is observed in our investigation.

Keeping in view the forgone discussion and in the light of current data available, it can be argued that the NORs are located on chromosomes in the form of secondary constrictions. The chromosomal formulations in the form of synaptonemal complexes (SCs) and recombination nodules (RNs) are observed to be involved in synapsis, chiasmata-formation and crossing-over in meiocytes of *Datura innoxia*. Our observations are further supported by Jimenez-Garacia *et al.*, (1989) who found similar results in *Allium cepa* meiocytes.

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