The Fine Structure of the Kinetochore in Meiotic Cells of *Tradescantia*

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Summary. Observations on *Tradescantia* cells in the second meiotic division revealed distinct regions in meiotic chromosomes. These areas 1) consistently stain less dense than the chromosomes themselves, 2) have direct connections with the chromosomes at certain points, and 3) serve as focal points for spindle tubules during meiosis. These lighter staining regions are similar in character to kinetochores (centromeres) found in animal cells.

Introduction

The fine structure of kinetochores has not been studied as extensively as that of other components of meiotic and mitotic cells. The reason for this appears to have been due mainly to the lack of a proper combination of fixation and embedding for preservation of the fine structure of these organelles.

Recently certain combinations of fixation and embedding, together with the mitotic inhibitor colcemid, have been successfully utilized to elucidate the fine structure of kinetochores in a mammalian cell (BRINKLEY and STUBBEFELD, 1966).

In this investigation similar procedures, without the use of colcemid, have been successfully employed to study the fine structure of kinetochores in a plant cell, the pollen mother cell of *Tradescantia* in the second meiotic division.

Materials and Methods

Cells undergoing meiosis in the stamen of *Tradescantia spec.* were observed under the light microscope, utilizing aceto-orcein squash preparations. When appropriate stages were found, the remaining cells were fixed for 1—2 hours in 4.0% glutaraldehyde, buffered at pH 7.3 with phosphate buffer. After the glutaraldehyde, the cells were rinsed for 5 min in buffer and subsequently fixed for 1 hour in 1.0% osmium tetroxide which was similarly buffered. All fixation was at room temperature. Following ethanol dehydration, cells were embedded in Epon 812. Thin sections were mounted on unsupported grids, double stained with uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope. All micrographs (except where specifically indicated) are from cells in prometaphase of the second meiotic division.

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Results

Kinetochores have been observed as early as prophase. These structures represent distinct regions on the chromosomes that stain less dense than the chromosomes themselves. Spindle tubules are first seen radiating from the kinetochores during prometaphase (Figs. 1 and 3).

Kinetochores occupy constricted regions along the chromosomes but they are not directly attached along their entire adjacent surface (Figs. 3 and 4, double arrows). Fig. 2 (an L-shaped chromosome) shows no direct connection between adjacent surfaces of the chromosome and kinetochore at $K_1$, further pointing out the restricted connection between the two structures. The L-shaped chromosome in Fig. 6 shows similar relationships.

Whether the chromosome-kinetochore attachment is an attachment at a single point or along a narrow strip extending the length of the kinetochore cannot be determined from the present observations.

Random sectioning through kinetochores shows a granular mass. The granulae have a diameter of 70—80 Å.

Spindle tubules can be seen to radiate from the kinetochores; however, they appear to penetrate less than one third the depth of the kinetochore (Figs. 4 and 5). This spindle tubule-kinetochore arrangement appears consistent throughout other stages that were studied (Figs. 7 and 8). Early in prometaphase some spindle tubules proximal to kinetochores have structural irregularities, e.g., increased diameters and lack of structural uniformity (Fig. 4, I). Spindle tubules have a diameter of 150—180 Å except in those instances where this irregularity exists.

Discussion

Studies on the fine structure of kinetochores are very limited. The major contributions reported so far (Brinkley and Stubbfield, 1966; George et al., 1965; Harris and Mazia, 1962; Nebel and Coulon, 1962) have all been directed toward kinetochores in animal cells. A single electron micrograph by Harris and Bajer (1965) is the only known work of this type on plant cells.

Prometaphase spindle tubules radiating from kinetochores indicate that these kinetochores may be physiologically active during prometaphase. The kinetochore as a center of organization of spindle tubule subunits has been reviewed by Mazia (1961) and Inoué (1964). The irregularity in some spindle tubules proximal to the kinetochore, found in the present study, may be related to the increased birefringence and initial orientation of tubular subunits described by Inoué in kinetochores at prometaphase. Spindle tubules are not static structures but are constantly in a dynamic state of flux (Inoué, 1964).