Cytochemical Electron Microscopic Studies on the Kinetochore of Pollen Mother Cells in *Tradescantia reflexa*

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The kinetochore is a conspicuous structure in dividing chromosomes, especially after metaphase, in mitotic and meiotic cells and it is believed to be important in movement of chromosomes towards the spindle poles. There are many reports of studies on the fine structure of the kinetochore, which have been reviewed by Luykx (1970) and Brinkley and Stubblefield (1970). Bajer and Molé-Bajer (1972) also wrote a critical review about structural details of the kinetochore.

Recently, electron microscopic studies on the kinetochore have shown that it contains DNA (Luykx 1970, Comings and Okada 1971, and Ross 1977), RNA (Braseltone 1975, Ross 1977, Bielek 1978, Espanda 1978, Rieder 1979a and 1979b) and basic proteins (Pardue and Gall 1970, Espanda 1978). These studies were mainly carried out on animal cells, such as those of the rat kangaroo (Ross 1977, Rieder 1979b), newt (Rieder 1979a), grasshopper and mouse (Espanda 1978), and HeLa cells (Bielek 1978): few studies have been made on the components of kinetochore of plant cells (Braseltone 1957, and Espanda 1978).

Therefore, in this work I examined the components of the kinetochore and their localizations in the plant, *Tradescantia reflexa*.

Material and methods

Pollen mother cells in the first meiotic division of *Tradescantia reflexa* were used as material. The cells were fixed in a mixture of 5% glutaraldehyde and 4% paraformaldehyde, adjusted to pH 7.0 with phosphate buffer, for 2 hours in a refrigerator. Then the cells were rinsed with the same buffer for 1 hour, and divided into four parts for the following treatment.

1) Conventionally fixation; The cells were post-fixed in 1% OsO4 adjusted to pH 7.0 with phosphate buffer, for 3 hours in a refrigerator. Then they were dehydrated in a graded series of acetone and propylene oxide and embedded in epoxy resin. Ultrathin sections were made and stained with lead citrate.

2) Ur-EDTA treatment: Cells were dehydrated with acetone and propylene oxide and embedded in epoxy resin. Ultrathin sections from the blocks were placed on coated with formvar membrane and stained with aqueous 5% uranyl acetate for 30 minutes at room temperature. Then the sections were treated with 0.2 M EDTA for 25 minutes in 35°C. Some specimens were prepared without EDTA treatment. Then the sections were rinsed with distilled water and stained with lead citrate.
3) RNase treatment and Ur-EDTA treatment: Cells were treated with 1 mg/ml of RNase for 3 hours at 37°C, and then processed as described in 2).

4) PTA-staining: Cells were dehydrated with acetone and then immersed in 1% phosphotungstic acid (PTA) in absolute acetone overnight in a refrigerator. They were then dehydrated in propylene oxide and embedded in epoxy resin. Ultrathin sections were made, but not stained.

The ultrathin sections made as described above were examined under an electron microscope, HU-12A.

Figs. 1-2. were obtained by the conventional method: 1, a pollen mother cell in the first meiotic anaphase. The upper and lower parts of the figure are polar regions. Homologous chromosomes are moving towards the poles. Arrows indicate kinetochores. 2, enlargement of the framed area in Fig. 1. The round kinetochore, which is somewhat less electron dense than the chromosomal arm, is present in a socket of the chromosomal arm. Kinetochore microtubules are seen. (Abbreviations of Figs. 1-7: CH, chromosomal arm; K, kinetochore; Mt spindle microtubules.)

Results

A cell in the first meiotic anaphase fixed in OsO₄ is shown in Fig. 1. The upper and lower parts of the figure are polar regions of the spindle body, and the homologous chromosomes are seen somewhat distant for the poles. Some chromosomes are cut through the kinetochore (Fig. 1, arrows). An enlargement of the kinetochore is shown in Fig. 2. The kinetochore (Fig. 2, K) appears somewhat less electron dense than the chromosomal arm (Fig. 2, CH) as a round protrusion of about 700 nm diameter. It consists of fine fibrils of about 20 nm width filled with amorphous material. These fine fibrils are continuous with the fibrils of the same width in the
Figs. 3-7. 3, ultrathin section stained with Ur-acetate and Pb-citrate from a cell fixed with a mixture of paraformaldehyde and glutaraldehyde. 4, chromosome after Ur-EDTA treatment. The spindle area is highly electron opaque. The periphery of the kinetochore is very electron opaque, while near the chromosomal arm it is less electron opaque. 5, chromosome obtained from a Ur-EDTA treated preparation after RNase-digestion. The chromosomal arm, kinetochore and spindle area are all less electron dense. 6 and 7: chromosomes obtained by PTA-staining. 6, chromosomes are electron opaque, but the kinetochore is less opaque than the chromosomal arm. Arrows indicate kinetochores. 7, transverse view of chromosome cut through the kinetochore. The kinetochore is in a socket of the chromosomal arm. Thin arrows show finger-like protrusions of the kinetochore. (Scales are all 1 μm)
chromosomal arm. This continuity suggests that the kinetochore fibrils contain DNA. The electron density of the kinetochore may be less than that of chromosomal arm because the chromatin fibrils are less densely packed. From the distal side (opposite side to the chromosomal arm) kinetochore microtubules (Fig. 2, Mt) extend towards the spindle pole. Although, kinetochore microtubules are seen between grooves around the ball-shaped kinetochore, their continuity with the kinetochore is not distinct.

Ultrathin sections of cells fixed only with aldehyde showed extremely low contrast images under the electron microscope. But, when these sections were then stained with aqueous 5% uranyl acetate, the images show high contrast (Fig. 3). The chromosomal arm (Fig. 3, CH) has high electron density, consisting of closely packed fine fibrils of 20 nm width, while, the kinetochore (Fig. 3, K) is seen as a mass of loosely packed fibrils of the same size as those in the chromosomal arm. The ends of kinetochore microtubules are seen between the strands consisting of these fibrils. In section treated with EDTA after staining with uranyl acetate, chromosomes (Fig. 4, CH) appear less electron opaque than the spindle area and cytoplams. However, only the periphery of the kinetochore (Fig. 4, K) on its distal side is electron opaque. In chromosomes of cells treated with Ur-EDTA after RNase, no electron opaque regions are seen around either the kinetochore or the chromosomal arm (Fig. 5, K). Bernhard (1979) reported that regions containing RNA, such as the nucleolus and ribosomes, remain electron opaque after uranyl staining and then EDTA treatment and that areas containing DNA are breached preferentially by EDTA. Thus the present results suggest that much of the kinetochore is occupied by materials containing DNA and that RNA, if present, is localized in the periphery of the kinetochore.

Chromosomes of cells stained with PTA showed high electron density, but the kinetochores were easily distinguished as less electron dense regions than the chromosomal arms (Fig. 6, arrows). This findings shows that the kinetochore contains basic proteins, because Sheridan and Barrnett (1969) reported that basic proteins, such as lysine- and arginine-rich proteins, are stained by alcoholic-PTA, although in this work PTA in acetone instead of alcohol was used. The difference in the electron opacities of the chromosomal arm and kinetochore suggests that they contain different amounts or types of basic proteins.

In transverse sections of the chromosome through the kinetochore, the kinetochore has a glove-like profiles (Fig. 7, K) and is seen in a socket of the chromosomal arm (Fig. 7, CH). On one side, the kinetochore adheres the chromosomal arm while its distal side shows fingers-like processes free from the chromosomal arm (Fig. 7, thin arrows). Kinetochore microtubules may terminate between these fingers-like protrusions, but unfortunately this could not be determined because microtubules were invisible in materials stained with PTA.

Discussion

There have been many recent reports on chemical components of the kinetochore. Brinkley and Stubblefield (1966) suggested that the kinetochore contains
RNA, and this idea was later supported by chemical studies (Braselton 1975, Ross 1977, Bielek 1978, Espanda 1978, Rieder 1979a and 1979b). In pollen mother cells of *T. reflexa*, I demonstrated that RNA is a chemical component of the kinetochore by preferentially staining technique of Bernhard (1969), and by RNase treatment, as shown in Figs. 4 and 5. Ross (1977) found that RNA is present in the outer layer of the kinetochore of HeLa cells. Rieder (1969a) also reported that the RNP component of the kinetochore is closely associated with the base of the kinetochore microtubules in lung cells of the newt. Rieder (1979b) concluded that RNA is localized in the inner layer of the trilaminar structure of the kinetochore in cells of the rat kangaroo. In pollen mother cells of *T. reflexa*, RNA seems to be present in the periphery of the ball-shaped kinetochore at the base of kinetochore microtubules, as shown in Figs. 4 and 5.

DNA was also concluded to be a component of the kinetochore from light microscopic (Lima-de-Faria 1958, Pardue and Gall 1970, MacGregor and Kezer 1971) and electron microscopic (Luykx 1970, Comings and Okada 1971) studies. Bernhard (1969) found that DNA-containing structures, chromonema, are breached by Ur-EDTA treatment. In the present material, much of the kinetochore was breached by Ur-EDTA treatment (Fig. 4). Moreover, the kinetochore contains the same type of fibrils of about 20 nm width as chromosomal arm, indicating that much of the kinetochore is formed of chromatin fibrils containing DNA.

Basic proteins have also been found in the kinetochore (Espanda 1978, Pardue and Gall 1970). In the present work also, much of the kinetochore was found of PTA-staining to consist of basic proteins coexisting with DNA and RNA, namely DNP and RNP (Figs. 6 and 7). In autoradiographic investigations on mouse, Pardue and Gall (1970) found that DNA in the kinetochore is present as heterochromatin. If this is so, the difference in the intensity of PTA-staining of the kinetochore and chromosomal arm in the present material may indicate difference in amounts and qualities of amino acids.

**Abstract**

Cytochemical studies were made on the kinetochore of chromosomes in the first meiotic metaphase and anaphase of pollen mother cells of *Tradescantia reflexa*. The kinetochore of *T. reflexa* is ball- or cup-shaped and about 700 nm in diameter. It seems to contain DNA, RNA and basic proteins judging from the effect of uranyle-EDTA treatment, RNase digestion and PTA-staining. The location of these chemical components were also examined.

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References


