Cell Division of Binuclear Cells Induced by Caffeine: Spindle Organization and Determination of Division Plane

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Synchronously dividing binuclear cells were induced in root tips of *Triticum turgidum* by caffeine treatment. Spindle and other microtubular configurations of such cells were studied using tubulin immunofluorescence and electron microscopy. The binuclear cells developed one, two or three prophase microtubule bands longitudinally, transversely or rarely in a cross configuration. During the mitotic entry binuclear cells formed prophase spindles separately around each nucleus. When the nuclei were located fairly apart, their spindle structures developed independently throughout all mitotic phases. But when the nuclei were located closely together their metaphase and anaphase spindles shared a common polar region. However, the two spindles in such cells retained their functional autonomy. They display structurally independent minipoles in the common polar region. After anaphase the neighbouring non-sister chromosome groups of nuclei divided by a common polar region. The peculiar mode of spindle and spindle polar region organization of binuclear cells and determination of the division plane in them are discussed.

Key words: Binuclear cells — Caffeine — Minipole — Phragmoplast — Preprophase microtubule band — Spindle polar region

The vegetative cells of angiosperms lack a structurally defined centrosome or a microtubule organizing center (MTOC) in the spindle polar region. However, this does not preclude them from undergoing well organized bipolar cell divisions, which are precisely ordered according to the morphogenetic requirement of the tissue. This is achieved by means of highly specialized microtubule (Mt) systems like prophase band (PPB), spindle and phragmoplast. The PPB is a morphological expression of the polarization of the cells and their commitment to divide on a predetermined plane. It forms in G2 period of the cells and disappears at late prophase. During prophase, spindle develops around the nucleus. It gradually assumes a bipolar form and is transformed into a metaphase spindle. After anaphasic segregation of chromosomes, the phragmoplast and the cell plate are formed in the overlapping region of the half spindles. The cell plate expands centripetally with the help of the phragmoplast and meets the parental wall at the region defined by the PPB (Rev. Baskin and Cande 1990, Gunning 1992, Lambert and Lloyd 1994). Thus, it is evident that the orderly progress of cell division in higher plants depends upon the spatial and temporal coordination of different Mt systems.

Due to the lack of a definite centrosome or MTOC the spindle polar region and spindle organization of angiosperm cells appear different from the astral spindle pole and spindle organization of animal cells. Due to the absence of such a definite structural entity, it has not been possible yet to study the nature of spindle polar region of angiosperm cells by micromanipulation, microbeam irradiation or by other means of dissecting them from the nuclear cycle or other aspects of the cell cycle. In this regard, binucleate cells are very useful models, because they provide systems in which two sets of mitotic apparatuses operate in a single cellular environment. Studies of dividing binuclear cells would elucidate how the different Mt structures—PPB, spindle and phragmoplast—associated with the two nuclei interact among themselves. Some recent studies done in binuclear fern protonemal cells induced by caffeine have revealed important information on cell division (Murata and Wada 1993a, b). In the present work we have investigated the Mt cycle of synchronously dividing caffeine-induced binuclear cells of *Triticum* root-tips paying particular attention to their mitotic and cytokinetic apparatuses. Nuclear cycle of such cells has been elaborately studied by some earlier investigators (Giménez-Martín et al. 1968, González-Fernández et al. 1971).

**Materials and Methods**

**Caffeine treatment**

Seedlings of *Triticum turgidum* were grown on moist filter paper layers for 3 days. Entire seedlings were treated with 5 mM caffeine for 2 hr by keeping them on filter paper layers moistened with the caffeine solution. After treatment they were washed several times with tap water and then incubat-
ed on water moistened filter paper for 14 hr. We chose a 14 hr post-treatment incubation period, because at this duration the root tip contained the highest population of synchronously dividing binuclear cells.

**Immunofluorescence of microtubules**

The root tips were fixed with paraformaldehyde, digested with cellulase and macerozyme, squashed on poly-L-lysine coated coverslips and extracted with Triton. The antibodies used were YOL 1/34 (Sera Lab) followed by FITC anti-rat (Sigma). DNA and cell wall staining were done with Hoechst and calcofluor white, respectively. Detail methods are described elsewhere (Panteris et al. 1991).

**Electron microscopy**

The excised root tips were prefixed with 2.5% glutaraldehyde with 1% tannic acid in 0.025 M phosphate buffer (pH 7) for 2 hr, washed thoroughly with phosphate buffer and post-fixed with 1% OsO4 in phosphate buffer for 3 hr at 4°C. The root tips were dehydrated with acetone series and embedded in Spurr’s resin. The semithin sections were stained with 1% Toluidine blue in 1% sodium tetraborate solution. The ultrathin sections were stained with uranyl acetate and lead citrate according to the standard schedule and examined under a Phillips 300 electron microscope.

**Results**

**Preprophase binuclear cells**

The nuclei of the binuclear cells formed by the present experimental method (2 hr caffeine treatment and 14 hr incubation in water) divided synchronously. Very rarely asynchrony was observed in prophase. The degree of asynchrony in such cells was also inconspicuous.

The general structure of the PPB in binuclear cells was similar to that of the PPB of normal *Triticum* root tip cells (Gunning and Sammut 1990, Gunning 1992, Panteris et al. 1995). However, variations were observed in their number and arrangement. Binuclear cells possessed either one, two or rarely three PPBs (Figs. 1–4, 11, 12). Likewise, they developed transversely or longitudinally along the axis of the cells (Figs. 1–3). The number of PPBs and their orientation were not related to the shape or size of the cell or the number of nuclei. Among the binuclear cells displaying one or two PPBs 46% showed two transverse (Figs. 11, 26c), 38% one transverse (Figs. 2, 12, 26b) and 16% one longitudinal PPBs (Figs. 1, 26a). The percentages have been derived from the account of 200 binuclear cells displaying transverse