Chromosome Polarization and Nuclear Rotation in *Allium cepa* Roots

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Since Pardue and Gall (1970) introduced *in situ* nucleic acid hybridization and differential Giemsa staining of chromosomes to demonstrate portions of chromosomes containing repetitive DNA, many other Giemsa staining techniques have been developed for demonstrating chromosome "banding" (Arrighi and Hus 1971, Drets and Shaw 1971, Sumner *et al.* 1971, Seabright 1971, Dutrillaux and Lejeune 1971, Yosida and Sagai 1972, Shiraishi and Yosida 1972, Dev *et al.* 1972, Kato and Yosida 1972). Most of the banding work utilizing differential Giemsa staining has been done on animal chromosomes. However, Vosa and Marchi (1972) used the technique of Sumner *et al.* (1971) to demonstrate differential Giemsa staining in the chromosomes of seven higher plant species. We have slightly modified the technique used by Vosa and Marchi (1972) in examining root tip chromosomes of *Allium cepa* (the common table onion). Our work has yielded data pertinent to chromosome polarization and nuclear rotation in this material.

Materials and methods

Although we used many different preparative procedures before Giemsa staining, the method reported here gave the best and most reproducible results.

Root tips were grown from *Allium cepa* bulbs suspended in tap water at room temperature. The roots were removed from bulbs and fixed in acetic-ethanol (1:3) for one hour. After fixation, two portions of the roots were removed for further processing. These portions included the first two millimeters of roots (the meristems) and four millimeter portions of roots removed one centimeter proximally from root apices. These portions of roots were hydrolyzed in 0.2N HCl at room temperature for 30 minutes. The HCl-treated portions of roots were then transferred to 45% acetic acid for approximately 10 minutes, after which the tissues were teased apart on slides in the same medium. The cells do not regain their turgidity in 45% acetic acid, and during squashing the cells seem merely to collapse (Fig. 1). The cover slips were removed by the dry ice method and the slides were flame dried. Flame drying seems to be necessary for the Giemsa stain to have access to the chromosomes. Presumably flame drying damages the cell walls and/or plasma membranes and allows them to become more permeable to the stain. Dried slides were placed in a saturated solution of barium hydroxide at 22°C for ten minutes followed by three 5 minute washes in distilled water. The slides were then incubated for one

hour in 60°C 2XSSC, after which the squashes were stained at room temperature with Giemsa stain (Harleco) diluted 1:9 in 0.12M phosphate buffer (pH 6.8). Excess stain was rinsed from the slides with distilled water and the slides were allowed to air dry. Dried squashes were mounted in Euparal. Comparable differential staining of telomeres also can be obtained using the technique of Drets and Shaw (1971).

In addition, root tips were fixed in Navashin's fixative for 24 hours, dehydrated, embedded in paraffin, and sectioned. Sectioned material was stained with iron hematoxylin and mounted in Permount.

Observations and photography were performed on a Leitz Orthoplan microscope equipped for Nomarski differential interference microscopy.

Fig. 1. Nomarski differential interference microscopic view of an unstained squash preparation of meristematic cells in a root tip of *Allium cepa*. Note the tendency for cells to remain in chains and for apparent daughter cells to remain in intimate contact. $\times 675$.

Results

Cells which have been treated with HCl, seem simply to collapse during squashing (Fig. 1). The telomeres of all of the chromosomes stain more or less distinctly (Figs. 2 and 3). The term *telomere* is used here only to indicate the extreme ends of chromosomes. There are only a few very tiny differentially stained regions on other portions of the chromosomes. This is surprising in view of the earlier reports by Levan (1946), Vanderlyn (1948), and Matagne (1968) of heterochromatin at the telomeres and centromeres of *Allium cepa* chromosomes. Apparently our stain-

Figs. 2-8. Differential Giemsa staining of telomeres from the meristematic zone of *Allium cepa* root tips. 2, metaphase in which most telomere polarization is lost. $\times 2000$. 3, anaphase showing establishment of the polarization of telomeres. $\times 2000$. 4, telophase when the anaphase polarization of telomeres is probably stabilized by attachment of the telomeres to the nuclear envelope. $\times 2000$. 5, interphase with obvious polarization of sister telomeres and an apparent lack of nuclear rotation. $\times 1120$. 6, interphase (left) and prophase (right) sister cells showing telomere and chromosome polarization and a lack of nuclear rotation. $\times 1120$. 7, prophase sister cells showing telomere and chromosome polarization and a lack of nuclear rotation. $\times 1120$. 8, interphase (bottom) and prometaphase (top) sister cells showing telomere and chromosome polarization and a lack of nuclear rotation. $\times 1120$.

ing technique reveals only a certain type of constitutive heterochromatin which is a conclusion reached by others who have used comparable staining on chromosomes of *Allium cepa* (Levan 1946) and other organisms (Yamasaki 1971, Cooper and Hsu 1972, Gropp and Natarajan 1972).

Figs. 9-12. 9, a chain of differentially Giemsa-stained nuclei from the meristematic zone of a root tip of *Allium cepa*. Polarization of telomeres is apparent, but nuclear rotation must have occurred in one or more of these nuclei since in none of these cells do the polarized telomeres of one cell face the polarized telomeres of an adjacent cell. $\times 1120$. 10, differentially Giemsa-stained cells from an elongated region of an *Allium cepa* root. $\times 235$. 11, 12, enlargements of the left and right nuclei, respectively, in Fig. 10, which have the same orientation in Fig. 10. Note the polarized telomeres to the left in Fig. 11 and to the top in Fig. 12. In such chains of elongated cells, nuclei typically show apparently random orientations of their polarized telomeres.

$\times 1120$.

Telomeres can be observed at any stage of the mitotic cycle including interphase (Figs. 2-8). So long as the nuclear envelope remains intact, telomeres are usually strongly polarized to one side of the nucleus in which they reside (Figs. 4-8). It appears that this polarization is a relic of the anaphase-telophase chromosome

orientation from the preceding division, which is maintained by attachments of the telomeres at least to the nuclear envelope.

An interesting aspect of HCl-treated squash preparations is that sister cells (cells derived from an immediately preceding mitotic division of a parent cell) remain in intimate contact whereas other cells are not nearly as likely to remain together (Fig. 1). Because of this, it is possible to determine whether nuclear rotation occurs in root tip cells. In order to determine whether a nucleus rotates one must have an outside reference point which has a fixed position in relation to the nucleus in question, and the nucleus in question must have a known orientation to the reference point at some known time. Furthermore, the nucleus in question must also have some marker in or on it which allows one to determine whether the nucleus has rotated with respect to the outside reference point. All of these criteria are met in sister cells in HCl-treated squash preparations. The outside reference point which has a fixed position in relation to the nucleus in question is the sister cell. The nucleus in question has a known orientation to the sister cell at a known time, i.e. telomeres in the nucleus face the sister cell at anaphase-telophase. Finally the stained telomeres of the nucleus in question provide a marker to determine whether the nucleus has rotated with respect to the sister cell. In the overwhelming majority of cases where sister cells seemed to be identifiable by their intimate association, the nucleus of neither cell appeared to have rotated from its anaphase-telophase position (Figs. 2-8). In only a few instances, out of many hundred meristematic pairs of sister cells examined, were there unequivocal cases of nuclear rotation (Fig. 9). We conclude that nuclear rotation is rare, but possible, in meristematic cells of onion root tips. In examining columns of cells from portions of roots taken one centimeter proximally from root tips (Fig. 10), we found that the telomeres maintain their grouping to one side of the nucleus (Figs. 11 and 12), but there is no longer any tendency for sister nuclei to have their telomeres' facing each other. It seems that at some point in these cells' differentiation or constantly in their differentiated state, nuclei of these non-mitotic cells must undergo nuclear rotation which results in an apparently random orientation of sister nuclei.

Discussion

Since the turn of the century there have been many reports based on visual observations and interpretations of radiation effects, which have suggested that chromosomes are polarized in interphase nuclei (Sax 1940, Levan 1946, White 1954, Evans and Bigger 1960, Evans 1961, Rhoades 1961, Kitani 1963, Fox 1966, Kumar and Natarajan 1966, Comings 1968, Wagenaar 1969, Fussell 1972). Such polarization is interpreted to be a remnant of the anaphase-telophase positioning of chromosomes which lasts through interphase. This report is a particularly clear substantiation of this generally accepted view of the interphase nucleus.

The subject of nuclear rotation has been largely ignored in higher plants, but the phenomenon has been well documented in many types of animal cells in tissue culture (for reviews see Leone *et al.* 1955, Puza 1965). Valcanov (1929, 1934) made extensive studies of nuclear rotation in Characeous algae where he found nuclei

may make complete rotations. In Vanderlyn's (1948) review of mitosis in the root tip of *Allium cepa* he maintains that nuclear rotation of meristematic cells is common rather than exceptional. From observations of sectioned *Allium cepa* roots stained with haematoxylin, Vanderlyn concludes that meristematic nuclei regularly rotate 180° from one division to the next. He cites Bělař (1928) and Heitz (1932) for supporting observations in *Tradescantia* and *Vicia*. Our haematoxylin-stained root tip sections do not allow this interpretation for *Allium cepa*. One does get the impression that nuclei may oscillate somewhat, but we could not see evidence of 180° nuclear rotation in this material. Nuclei that go into division with their axes tipped sufficiently from the typical parallel orientation with the root axis may contribute the occasional periclinal divisions that occur in the root meristem. In contrast, our HCl-treated material gives far clearer and more interpretable data on nuclear rotation, and non-rotation of nuclei seems to be the rule. Non-rotation is probably an expression at the cellular level of one aspect of the polarity of the root meristem. Non-rotation of nuclei may be one means of determining that continued cell divisions occur perpendicular to the long axis of the root. Apparently at some time after the cells stop dividing the nuclei become free to rotate and sister nuclei lose any particular orientation to each other. It may be of significance in generalizing the concept of non-rotation of plant meristem nuclei that in Andrew Bajer's extensive observations of mitosis in *Haemanthus* endosperm, he has not observed nuclear rotation (personal communication).

Summary

By using an *in situ* DNA denaturing-renaturing technique for differential Giemsa staining of root chromosomes of *Allium cepa*, telomeres were specifically stained. In meristematic cells, the position of the telomeres intranuclearly indicates that chromosomes remain polarized from anaphase through prophase of the following division and that nuclear rotation is rare during this time. In contrast, non-mitotic cells one centimeter proximal to the root tip also retain chromosome polarization, but the nuclei apparently are capable of rotation. We suggest that non-rotation of nuclei is one aspect of the polarity of the root meristem.

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