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Oud, J.L.; Nanninga, N.

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The relation between cell size, chromosome length and the orientation of chromosomes in dividing root cortex cells

J.L. Oud and N. Nanninga

BioCentrum Amsterdam, Institute for Molecular Cytology, University of Amsterdam, Plantage Muidersgracht 14, NL-1018 TV Amsterdam, The Netherlands

Key words: cell size, chromosome length, chromosome orientation, *Crepis capillaris* root cortex, *Vicia faba*

Abstract

Cells in the root meristem are organised in longitudinal files. Repeated transverse cell divisions in these files are the prime cause of root growth. Because of the orientation of the cell divisions, we expected to find mitoses with an spindle axis parallel to the file axis. However, we observed in the root cortex of *Vicia faba* large number of oblique chromosome orientations. From metaphase to telophase there was a dramatic increase of the rotation of the spindle axis. Measurements of both the size of the cortex cells and the chromosome configurations indicated that most cells were too small for an orientation of the spindle parallel to the file axis. Space limitation forced the spindle into an oblique position. Despite this spindle axis rotation, most daughter cells remained within the original cell file. Only in extremely flat cells did the position of the daughter nuclei forced the cell to set a plane of division parallel to the file axis, which result in side-by-side orientation of the daughter cells. Telophase spindle axis rotations are also observed in *Crepis capillaris* and *Petunia hybrida*. These species have respectively medium and small sized chromosomes compared to *Vicia*. Since space limitation, which causes the rotation, depends both on cell and chromosome size, the frequency and extent of the phenomenon in former two species is comparatively low.

Introduction

The number and orientation of cell divisions, as well as cell elongation and differentiation, determines the tissue organisation of a root. Since a root is a tubular structure, three principal orientations of cell divisions can be distinguished: periclinal, radial and transverse (Fig. 1). In the first two classes the axis of the mitotic spindle is oriented perpendicular to the root axis (respectively parallel to the radius and the periphery of the root). Cells which divide transverse show a spindle axis parallel to the root axis. The stem cells for root development are located in the so-called quiescent center. Descendants of the infrequently dividing quiescent center cells ('initial' cells; Barlow, 1978) give rise to the root cap tissue and the various cell layers (for review see Clowes, 1976; Barlow, 1984). According to Luxová (1975) the formation of the layers (epidermis, cortex, stele etc.) is the result of periclinal divisions, whereas anticlinal divisions increase the number of cells in an individual layer, and transverse divisions

(followed by cell elongation) are responsible for the longitudinal growth of the root. All three types of cell division can be found in the formative part of the root meristem (Fig. 1). Cell divisions in the proliferative part are almost exclusively transverse. As a result, the cells in the latter part are organised in longitudinal files, parallel to the root axis.

Looking at dividing cells in the proliferative part of the meristem in *Vicia faba* roots, we observed an unexpected large number of oblique anaphases and telophases, apparently caused by a rotation of the spindle axis (Oud and Nanninga, 1992). Oblique chromosome orientations are also observed in the root meristem of *Crepis capillaris* and *Petunia hybrida*. The analysis of three-dimensional confocal microscopy images of root cortex cells, showed that the degree of spindle rotation is related to both the size of the dividing cells and the length of the chromosome arms. The spindle is forced to rotate if the cell height is insufficient for a full separation of the two sets of chromatids during anaphase and telophase. Oblique telophase orien-

Fig. 1. Schematic representation of a root tip with an indication of the three principal orientations of a cell division (drawn as anaphases). The root cap and the different cell layers originate from cells in the quiescent center. The present study is restricted to dividing cells in the proliferative part of the meristematic region of the cortex (grey cells in the side view).

tations influence the position of the nuclei in daughter cells; they are shifted away from the center towards opposite directions. In spite of the spindle axis rotation and the displacement of the nuclei, the relative position of the daughter cells is nearly always unaffected, i.e. on top of each other, contributing to a further longitudinal growth of the cell file.

Material and methods

Vicia faba, *Crepis capillaris* and *Petunia hybrida* plants were grown under standard conditions in flowerpots in a greenhouse. Lateral roots of 3 to 4 weeks old plants were fixed in ethanol/glacial acid (3:1, v/v) for 30 min. *Vicia* and *Petunia* material was embedded in a 2:1 (v/v) mixture of polyethyleneglycol (PEG) 1500 and PEG 4000. Further details about the embedding technique are given by Oud and Nanninga (1992). 20 to 25 μm thick sections of the PEG embedded roots were stained in a 50 $\mu\text{g mL}^{-1}$ McIlvaine (pH 7.0) buffer (+ 5 mM MgCl_2) solution for 15 min. at room temp., and mounted in glycerine to which 2% DABCO antifade (Sigma D 2522) was added. The *Crepis* roots were macerated and stained according to the standard Feulgen procedure (Oud et al., 1989). The preparations were analysed by confocal microscopy (for technical details see Brakenhoff et al., 1990 and references therein). Depending on the image, voxel sizes of 200 to 400 nm lateral, and 350 to 700 nm axial were used. The 3-D images consisted of 24 to 36 optical sections. For the analysis of the confocal images a HP 425T work-

station and Scilimage software (Kate et al., 1990) were used. Solid model like images, as depicted in Figures 2 and 6, were made with the help of a ray-tracing technique, combined with an artificial shadowing to enhance the impression of depth (so-called FSP algorithm; van der Voort, 1989). The IMAP3D program (Houtsmuller et al., 1992; Montijn et al., 1994) was used to visualise simultaneously the XY, XZ and YZ aspect of 3-D images, to measure cell sizes, and to determine the orientation of file and spindle axes. From repeated measurements we estimated an accuracy of approximately $\pm 0.5 \mu\text{m}$ for length measurements and $\pm 2^\circ$ for angle measurements.

Results

Chromosome orientation in *Vicia faba* cortex cells

The analyses are based on three-dimensional images (i.e. series of optical sections), obtained by confocal microscopy, of 20–25 μm thick sections, stained with the DNA specific fluorochrome mithramycin. An example of a computer processed 3-D image is shown in Figure 2. The 3-D images enabled the analysis of 4 samples of 50 cells each, of respectively metaphase, early anaphase, late anaphase and telophase. The discrimination between early and late anaphase was based on the degree of separation of the two sets of chromatids. A cell was classified as late anaphase as soon as all chromatids were fully separated.

To compare the chromosome orientation in relation to the file axis, we constructed on the computer screen a line through each chromosome complement in such a way that it represented the most likely orientation of the mitotic spindle axis (as indicated in Fig. 2). A comparable procedure was used to define the axis of the cell file, whereafter the angle between the spindle axis and the file axis was calculated. There proved to be no preference for the direction of the rotation; on the average there were as many clockwise as counter-clockwise rotations; also in the case of several oblique orientations in the same file of cells. To facilitate the comparison of the subsequent stages of mitosis, we divided all measurements in three classes, comprising of angles of respectively $\leq 5^\circ$, 5° to 20° and $\geq 20^\circ$. The result of the comparison is visualised in Figure 3. These arbitrarily chosen classification gives a good impression of the percentage of cells with a chromosome parallel to the file axis (as expected in a transversally dividing cell), and the cells with moder-

Fig. 2. 3-D image of a part of two files of cells in the *Vicia faba* root cortex. The images are based on stacks of optical sections obtained with confocal microscopy and processed with the SFP algorithm. ↔: orientation of the file axes; →: orientation of the anaphase spindle axis; *: pairs of nuclei which are shifted diagonally out of the center of the cell, towards opposite directions. Note the rotation of the spindle axis with respect to the file axis. The bar represents 10 μm .

Fig. 3. The deviation of the spindle axis with respect to the axis of the cell file. The analysis is based on 4 samples of 50 each, respectively in metaphase, early and late anaphase and telophase. The data are grouped in three classes, which correspond with non-rotated, moderately rotated and severely rotated spindle axes. Note the strong increase in spindle rotation from metaphase to telophase.

ate or severe deviations from the expected orientation. The early anaphase in Figure 2 is characterised by an approximately 40° rotation of the spindle axis. As can be seen in Figure 3, there is a dramatic shift in the percentage of cells with a 20° or more rotation from metaphase to telophase. Two-thirds of all metaphases are aligned (almost) parallel to the file axis, whereas three-quarters of all telophases showed a significant rotation.

Fig. 4. The mean size of a dividing *Vicia faba* root cortex cell, compared with the mean size of early and late anaphase and telophase chromosome orientations. For the sake of clarity, the equator plane is indicated in the schematic representation of the metaphase. In an average sized cell there is only enough space for a full separation of the anaphase and/or telophase chromatids, unless the spindle axis rotates towards an orientation parallel to one of the diagonals (broken lines b, c and d).

Since there proved to be a relation between the orientation of the chromosomes and the size of the dividing cortex cells (see discussion), we also measured cell sizes. Root cortex cells have a rather rectangular shape; therefore the sizes are measured as cell height, width and depth, corresponding to the edge parallel to, respectively, the longitudinal axis, radius and periphery of the root. As shown in Figure 4, the mean height of dividing cells in the proliferative region of the *Vicia faba* root cortex proved to be 19 μm (with a range from 8 μm to 32 μm). The mean cell width was 24 μm (20–27 μm) and the mean cell depth was 15 μm (12–19 μm). In addition, we also measured the size of the chromosome configurations. The total longitudinal size of the two sets of chromatids of anaphase and telophase (see Fig. 4) increased from an average 19 μm (16–22 μm) in early anaphase to 25 μm (23–26 μm) at the end of telophase.

The metaphases with a moderately rotated spindle axis (14 cells in a sample of 50 cells) and the 2 cells which showed a more than 20° rotation invariably were cells with a severely reduced space. One of the latter two cells is schematically depicted in Figure 5. This cell was the smallest dividing cell in all samples, only being 8 μm high. A transverse chromosome orientation is not possible in such a 'flat' metaphase since most of the *Vicia* chromosomes are too long to be fully positioned in the metaphase equator plane. The centromere region of each of the chromosomes is oriented in this plane but a larger part of the remaining chromosome is oriented more or less perpendicular to the equator plane (see the side views in Fig. 5). Besides the observed oblique orientation, also a periclinal or a radial orientation would be possible. However, they

Fig. 5. The orientation of the metaphase equator plane in an extreme flat cell (left), and the three principal metaphase chromosome orientations drawn as side views of an equally sized cell (right). Only some of the chromosomes are drawn. The centromeres (black dots) are oriented in the equator plane (grey), whereas larger parts of the chromosome arms stick out of the plane. Also in other, comparable flat, dividing cells in the proliferative part of the cortex meristem, periclinal or radial orientations are never observed.

were never observed in the few examples of extreme flat dividing cells.

The position of the nuclei in daughter cells

Besides the chromosome orientation in dividing cells, we also took notice of the position of the nuclei in interphase nuclei. In most cell files pairs of small, somewhat flattened, interphase nuclei can be seen which are often shifted out of the center of the cell, towards opposite directions (in Fig. 2 two of these pairs are indicated by an asterisk). In view of the relatively small size of these cells and their nuclei, they probably represent young G₁-phase nuclei. The much larger S- and G₂-phase nuclei (Fig. 2: left file of cells) are nearly always positioned in the center of the cell. Apparently, the increase in nuclear size during interphase also results in a less eccentric position. The significance of these 'shifted' G₁-phase nuclei will be discussed in the next section.

*Chromosome orientation in *Crepis capillaris* and *Petunia hybrida**

As described above, the orientation of the chromosomes in dividing cortex cells has been analysed extensively in *Vicia faba*. Roots of *Crepis capillaris* and *Petunia hybrida* have been studied to verify whether the phenomena found in *Vicia* also occurred in other species. An example of two dividing *Crepis capillaris* root cortex cells is shown in Figure 6. The equator plane of the metaphase has an orientation (as expected)

perpendicular to the file axis, whereas the late anaphase shows a 27° rotation of the spindle axis. Among others, the three species are different with respect to the size of the chromosomes. The relevant data for the three species are summarised in Table 1. The mean cell height proved to be almost the same in the three species, in spite of the considerable differences in chromosome size. As expected, the differences in length of the longest chromosome arm (12 μm for *Vicia* to 5 μm for *Petunia*) have consequences for the size of the ana- and telophase complements (25 μm for *Vicia* to 17 μm for *Petunia*). The estimate of the percentages of cells with an at least moderate spindle axis rotation (90% for *Vicia*, 50% for *Crepis* and 15% for *Petunia*) is roughly in accordance with the differences in cell and chromosome size. Both in *Crepis* and *Petunia* we also observed pairs of daughter cells with shifted interphases nuclei.

Discussion

The relation between cell size and spindle axis rotation

In the proliferative part of the root cortex meristem we expected to find dividing cells with a spindle axis parallel to the file and root axis. Such transverse divisions contribute to the elongation growth of the root by increasing the number of cells in the files (see Fig. 1). Surprisingly, we observed that most dividing cells showed oblique orientations of the anaphase and telophase chromosomes. The relation between cell size (especially cell height) and chromosome orientation proved to be a key factor in the understanding of these oblique orientations, as illustrated for *Vicia faba* in Figure 4. The measurements show a considerable discrepancy between the mean cell height (19 μm) and the mean size of a telophase configuration (25 μm). These figures indicate that in an average cell there is not enough space for a full upright (i.e. parallel to the file axis) separation of the chromatids during anaphase and telophase. As a consequence, in a majority of cells (90%) the spindle axis is forced to rotate towards one of the edges during anaphase. To give an impression of the resulting gain in space, the mean size of the different cell diagonals is also shown in Figure 4 (dotted lines b, c and d.). Only cells with a height of at least 25 μm are large enough to permit the expected non-rotated positioning of the two set of chromatids.

The relation between cell size and spindle axis rotation has been studied in material fixed in 3:1 ethanol

Fig. 6. Confocal microscopy based 3-D image of a metaphase and anaphase cell in the root cortex of *Crepis capillaris*. As shown in the accompanying drawing, the spindle axis of the anaphase is 27° rotated compared to the file axis, whereas the metaphase equator plane is oriented in an almost non-rotated position perpendicular to the file axis.

Table 1. Mean cell size, chromosome length and percentage of oblique orientations in root cortex cells of three plant species

Species	Mean cell height	Length of longest chromosome arm ^a	Mean total length of telophase complement ^b	% of telophases with $>5^\circ$ spindle axis rotation ^b
<i>Vicia faba</i>	19 μm	12 μm	25 μm	90
<i>Crepis capillaris</i>	19 μm	8 μm	22 μm	50
<i>Petunia hybrida</i>	18 μm	5 μm	17 μm	15

^ameasured in 10 metaphases (not treated with a spindle inhibitor);

^bestimate based on respectively 50, 25 and 25 telophases.

acetic acid. In this respect it is relevant to consider the possibility that the observed phenomena are mainly the result of cell shrinkage by the fixative. There are however several reasons why this is to our opinion rather unlikely. First, if there is a significant shrinkage of the cells one would expect a deformation or compression of the chromosome and spindle configuration rather than a rotation. Second, it is unclear why there is such a difference in the degree of shrinkage, resulting in a considerable range in rotation. And finally, we have recently observed oblique anaphases, telophases and cell plates also in preparations for electron microscopical studies, which were fixed with 3% glutaraldehyde and 2% formaldehyde. One of the consequences of a spindle axis rotation is an eccentric position of the nuclei in the daughter cells. Two examples of G₁-phase nuclei which are shifted to opposite directions

can be seen in Figure 2. Although, three-quarters of all telophases show a considerable spindle axis rotation (Fig. 3), which results in these 'shifted nuclei', the daughter cells are nearly always positioned on top of each other, sharing the same cell file. We observed only rarely that daughter cells are positioned side-by-side (comparable to the result of a radial or periclinal cell division). In other words, even with a spindle axis rotation the cell divisions remain essentially transverse, although the plane of division is often no longer perpendicular to the file axis, but sigmoid shaped or oblique (for details about this aspect see Oud and Nanninga, 1992).

Only in extremely flat dividing cells, as the one depicted in Figure 5, an extreme oblique (i.e. almost vertical) cell wall seems to be inevitable. A more transverse wall will intersect the daughter nuclei. It

is expected that the result of the division of an extreme flat cell is a side-by-side positioning of the daughter cells. The low frequency of such flat cells (approximately 2% of all dividing cells) is in accordance with the rather exceptional observation of a file of cells with one pair of cells next to each other instead of on top of each other (Oud and Nanninga, 1992).

The above described model, in which cell size is responsible for the spindle axis rotation and indirectly determines the position of the daughter cells, is in accordance with the concept of the critical aspect ratio as proposed by Barlow and Adam (1989). They found in the root cortex of tomato that 'tall' cells (higher than wide) divide transversally, whereas 'flat' cells (wider than high) divide periclinally. The transition between flat and tall lies in the range of 1.9 to 2.3.

Oblique chromosome orientation in relation to chromosome size and cell type in various plant species

Spindle axis rotations occur very frequently, but not exclusively in *Vicia faba* root cortex cells. We observed oblique orientations and pairs of shifted interphase nuclei also in the same tissue of *Crepis capillaris* and *Petuni hybrida*. In these cells, the frequency and degree of rotation seems to be related to the size of the chromosomes of the species. Of the species mentioned above, oblique orientations are most abundant in *Vicia*, are found only occasionally in *Petunia*, whereas *Crepis* takes a position in between. This order roughly corresponds with the mean size of the chromosomes. A remarkable point which emerges from these findings is that, at least for root cortex cells, there is obviously no relationship between the size of the cells and the chromosome. However, there are large differences in cell shape between cell types. Therefore oblique chromosome orientations occur much more frequently in one tissue compared to another. In *Vicia* for example, they are almost absent in the root stele region, in which the cells are considerably longer compared to the more cubical or flat cells in the cortex. The occurrence of spindle axis rotations in other plant tissues is among others reported in stamen and leaf cells of *Tradescantia virginica* (Bělař, 1930) and in guard mother cells of *Allium cepa* (Palevitz and Helper, 1974). Also in these studies it is concluded that cell size limitations are the principal cause of the observed oblique orientations.

Further research

Further research with respect to the relation between cell size and spindle axis rotation during cell division is focused on the role of the cortical microtubules in general and that of the pre-prophase band of microtubules (PPB) in particular. It is generally believed that the PPB, which is visible from G₂-phase to early prophase, predicts the site of the subsequent cell division (Gunning and Wick, 1985; Williamson, 1991 and references therein). If a cell is able to sense its dimensions prior to cell division, we have to expect the occurrence of oblique PPBs in relative flat cells. On the contrary, fully transverse PPBs in flat cells, should point to the existence of a mechanism to adjust the plane of division from transverse to a sigmoid or oblique shape. In this respect, current studies by De Ruijter (Dept. of Plant Cytology and Morphology, Agricultural University Wageningen, the Netherlands) have not given so far an indication for the occurrence of clear oblique PPBs in flat *Vicia faba* root cortex cells (personal communication; details about this study will be published elsewhere).

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